THESIS

THE USE OF A PHOTOELECTRIC COLORIMETER IN THE MICRODETERMINATION OF FLUORINE

THE MICRODETERMINATION OF IODINE IN CEREAL GRAINS OF ALBERTA

by

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This is to certify that the undersigned have read and recommend to the School of Graduate Studies for acceptance, this thesis submitted in partial fulfilment of the requirements for the degree of Master of Science.

Professor

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Submitted in partial fulfilment of the requirements for the degree of Master of Science.

by

James F. A. Murphy
Under the direction of Dr. O. J. Walker

Time devoted to thesis work was 5.3 out of 7.0 months or three full courses, counting four courses as one academic year's work.

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THE USE OF A PHOTOELECTRIC COLORIMETER IN THE MICRODETERMINATION OF FLUORINE

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Introduction

It has been established (4, 10, 11) that "dental fluorosis" or mottled enamel is associated with the fluorine content of water. The mottling is produced during the period of development in which the teeth are calcifying in the gums (4). The fluorine content of water is therefore of some medical importance. The methods of fluorine (fluoride) determination may be divided roughly into titration methods (1, 2, 3, 15, 16) and colorimetric methods based on the extent of fading of a colored compound caused by the fluoride ion. One colorimetric method, developed by Sanchis (9) depends on the bleaching effect of fluorides on the lake formed by sodium alizarin sulphonate and zirconyl nitrate. It is a standard method that is widely used because of its accuracy and speed.

Underlying Theory of Colorimetry

The term colorimetry as used here includes measurement or comparison of color to determine the amount of some constituent of a solution. The constituent to be determined must be colored, react with a suitable reagent to give a color or as in the case of fluorine cause a change in color of one of the reagents.

Solutions used in colorimetry exhibit color as a result of reflection or transmission of unabsorbed radiant energy. Comparison of the colored system containing the unknown constituent with a similar system containing a known amount of that constituent is the basis of chemical colorimetry. Instruments used are therefore more correctly called comparators. Four general methods are used to compare colors.

Standard Series Method

A series of standards is used. The depths of the solutions in the standard and unknown are the same. The unknown is compared visually with each member of the series until a match is found. The concentration of the desired constituent in the unknown is then the same as that in the standard which it matches.

Duplication Method

The unknown is placed in a suitable vessel and the upper level of the solution is brought to a given mark. In a similar tube the color forming reagents are added and solvent added to just less than the volume of the unknown. Then a standard stock solution of the constituent being determined is added from a buret until, when the level is brought up to the mark, the color in the two tubes match. The unknown then contains the same amount of the constituent as was added to the comparison tube.

Dilution Method

The volumes of the standard and unknown are adjusted by adding solvent to the more concentrated solution to obtain a visual match. It is assumed in this method that the concentration of the color-forming constituent is proportional to the depths (volumes) of solution observed.

Balancing Method

The color of the unknown solution is matched with a standard by adjusting depths of solution. The concentration of the desired constituent in the two solutions must be inversely proportional to the depths of solution.

The filter photometer is another instrument used to compare the light-absorbing properties of a colored system. The use of the instrument requires some knowledge of the color characteristics of the solution to be measured and the applicability of the laws of light absorption.

Bouguer's Law states that each layer of equal thickness absorbs an equal fraction of the light which traverses it. The absorption varies directly as the logarithm of the thickness. If a layer of unit thickness transmits a fraction "t" of the light incident upon it then a thickness "b" will transmit the fraction "t^b". and $I = I_o\, t^b$ where I_o and I are the incident and transmitted light respectively. The law may also be expressed in the form $T = e^{-k'(b)}$ where "T" is the transmittancy (I/I_o), "e" is the base of natural logarithms and "k'" is the absorption coefficient $(-\log_e t)$.

Beer's Law involves the relationship between absorptance and concentration of solute in solution. The law states that the absorptance of the solution is directly proportional to the concentration of the solute. If "t" is the transmittancy for a given thickness of solution of unit concentration then the transmittancy "T" for the

same thickness of a solution of concentration "c" is given by "T" = t^c. The law applies if the light used is approximately monochromatic and the nature of the light absorbing particles does not change with concentration. For a thickness "b" and concentration "c" the laws above are combined in the expression $T = t^{bc}$ (with "t" the transmittancy for a system of unit concentration and thickness). This may be written in the form

$$T = e^{-k'(bc)}$$

but $T = I/I_o$ so $I = I_o\, e^{-k'(bc)}$

then $\log_{10} \frac{I_o}{I} = kbc \quad (k' \neq k)$

$= D$ (optical density)

and $C = \frac{\log_{10} I_o/I}{kb}$

"k" above is designated as the extinction coefficient.

Solutions conforming to Beer's Law have a constant molecular extinction coefficient at all dilutions and thicknesses for a given wave length. Spectrophotometric curves (extinction coefficient vs. wave length) for a solution obeying Beer's Law should have the same shape for different concentrations and in addition the extinction coefficients should be proportional to the concentrations. A curve from a spectrophotometer will have a maximum at

the wave length of maximum absorptance* of the system. At this point there is the largest change in extinction coefficient (transmittancy*) for a given change in concentration. Thus the transmittance-concentration curve at this wave length will have the best slope.

The purpose of the research reported in this section of the thesis is to adapt a photoelectric colorimeter (a filter photometer) to the Sanchis method for the determination of fluoride and to compare results from this method with those obtained by the visual Sanchis method.

Discussion of Colored System to be Used

The fading effect of the fluoride ion on the red lake formed by combination of sodium alizar in sulphonate and zirconyl nitrate is due to the formation of complex ions between the zirconium and fluoride ions. The sodium alizarin sulphonate released from combination is yellow in color in the acid solution. Thus with increasing amounts of fluoride the color of the solution changes gradually from the red of the lake to the yellow

* Terms conform to the recommendations of the report of the Spectrophotometry Committee of the Optical Society of America.

of the sodium alizarin sulphonate. The zirconium-fluoride complexes are not colored. Venables (13) reports that the following complexes occur:

$Zr_3F_{17}^{-5}$, $Zr_3F_{13}^{-5}$, ZrF_5^{-} , $ZrF_6^{=}$, $ZrF_7^{\equiv}$

In dilute solutions complexes containing much less fluorine may occur.

To assist in the choice of optimal wave lengths and concentrations a series of spectrophotometric curves were obtained on the red zirconium alizarin lake and the red and yellow forms of sodium alizarin sulphonate. This latter compound is red in neutral and yellow in acid solutions. The filters are used in the photoelectric colorimeter to block off all light except a narrow band in the region of maximum absorptance. This increases the change in transmittancy for a given change in concentration when the colorimeter is in use.

Preliminary Experimental Work

A Hilger-Nutting, visual spectrophotometer was used to obtain curves for solutions of sodium alizarin sulphonate in distilled water of the following concentrations:

1 7.0 mg. per 100 ml. of solution

2 8.5 mg. per 100 ml. of solution

3 14.3 mg. per 100 ml. of solution.

A description of the instrument is given in Gibb: "Optical Methods of Chemical Analysis" pp.79 - 81. The extinction coefficient which is given by the expression $k = \log_{10} \frac{I_o}{I} / bc$ is read directly from the instrument. The curves obtained by plotting the extinction coefficient against the wave length in millimicrons for the above solutions are shown in Figure (I). The data from which these curves were plotted are given in Table I.

Acid solutions of sodium alizarin sulphonate of the following concentrations were also examined.

4 4.3 mg. per 100 ml. of solution

5 8.5 mg. per 100 ml. of solution

6 17.0 mg. per 100 ml. of solution

The solutions were acidified with 2.0 ml. of 2.95 N hydrochloric and 2.0 ml. of 4.36 N sulfuric acids per 100 ml.

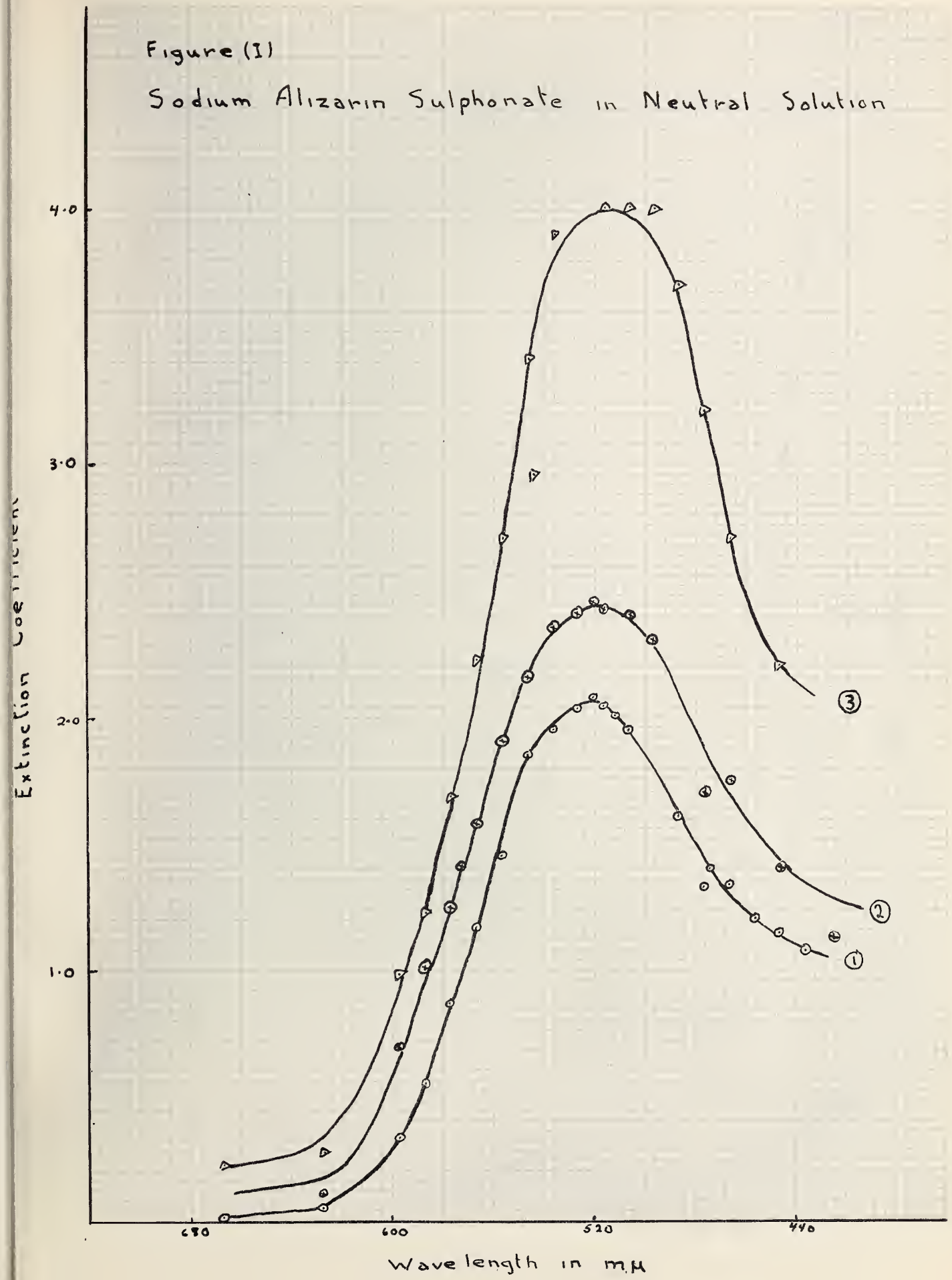
The data for solutions 4, 5 and 6 are presented in Table I and plotted in Figure (II).

TABLE I

Wave length millimicrons	Extinction Coefficients					
	Sol.1	Sol.2	Sol.3	Sol.4	Sol.5	Sol.6
665	0.01		0.22			
625	0.04	0.10	0.27	0.09		0.04
595	0.34	0.70	0.98		0.11	0.07
585	0.55	1.0	1.22	0.21	0.15	0.09
575	0.86	1.24	1.68	0.17	0.17	0.10
565	1.16	1.57	2.22	0.10	0.20	0.10
555	1.44	1.90	2.7	0.16	0.21	0.12
545	1.85	2.15	3.4	0.17	0.24	0.16
535	1.95	2.35	3.8	0.06	0.25	0.07
525	2.03	2.40	3.9	0.07	0.27	0.03
520	2.07	2.45				
515	2.04	2.42	4.0	0.09	0.29	0.15
510	2.00					
505	1.95	2.40	4.0	0.10	0.35	
495	1.90	2.30	4.0	0.06		0.30
485	1.60	1.95	3.7	0.06	0.38	0.55
475	1.33	1.65	3.2	0.17	0.60	0.90
465	1.33	1.75	2.7	0.215	0.83	1.40
455	1.20		2.4	0.27	1.05	2.15
445	1.15	1.20	2.20	0.31	1.40	3.0
435	1.08			0.36	1.85	3.9
425				0.44	2.4	>4

Figure (I)

Sodium Alizarin Sulphonate in Neutral Solution



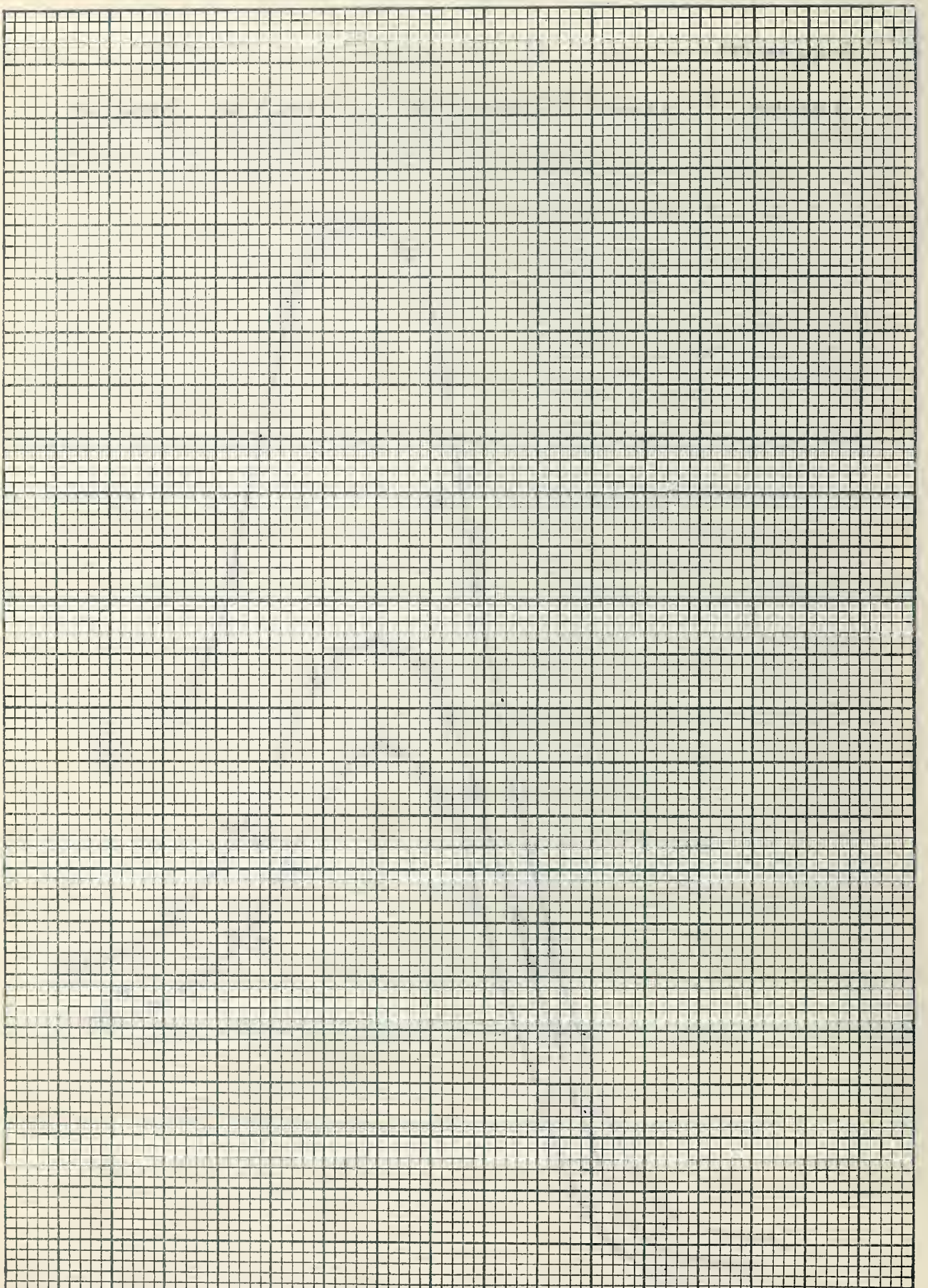
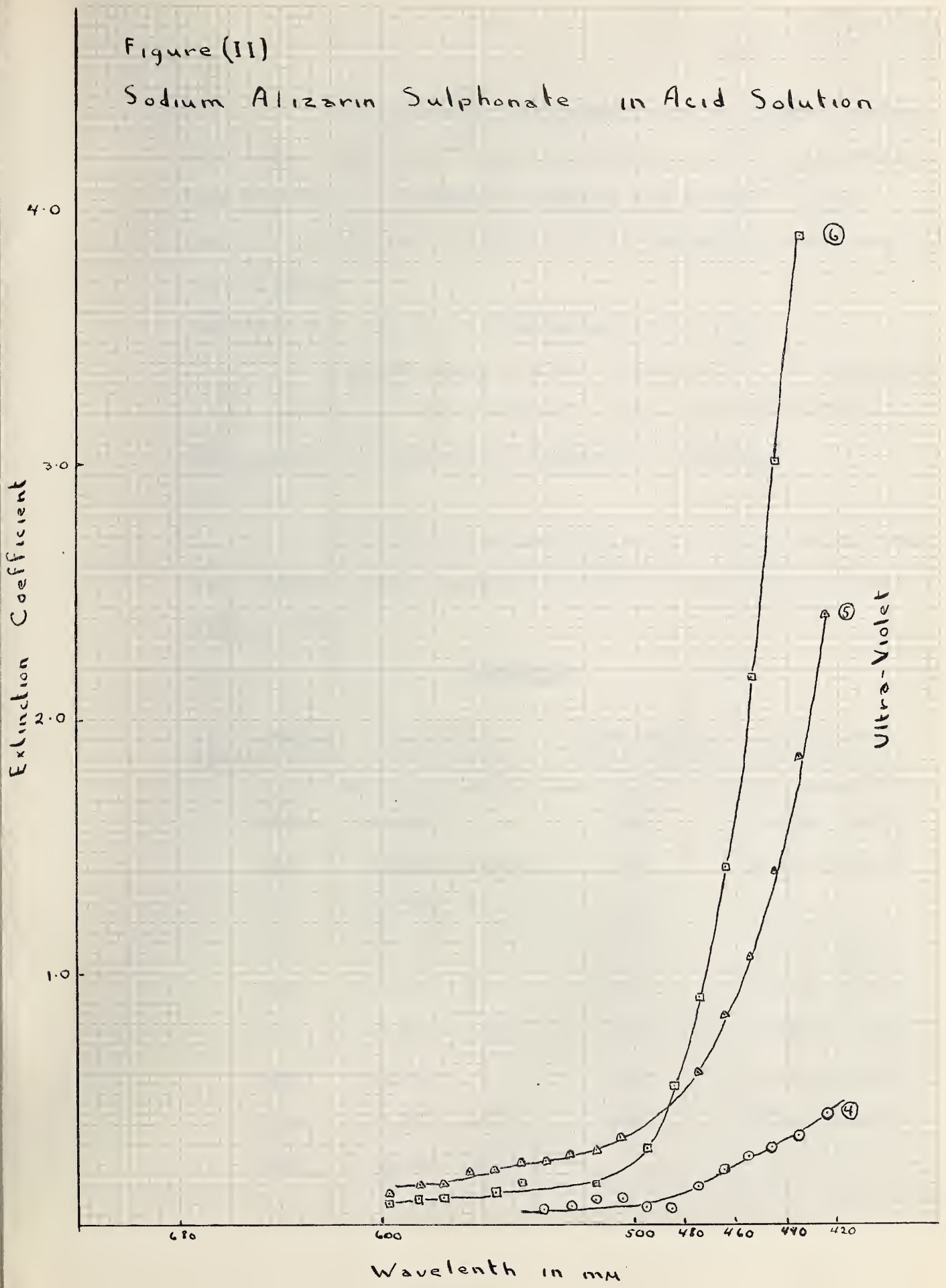
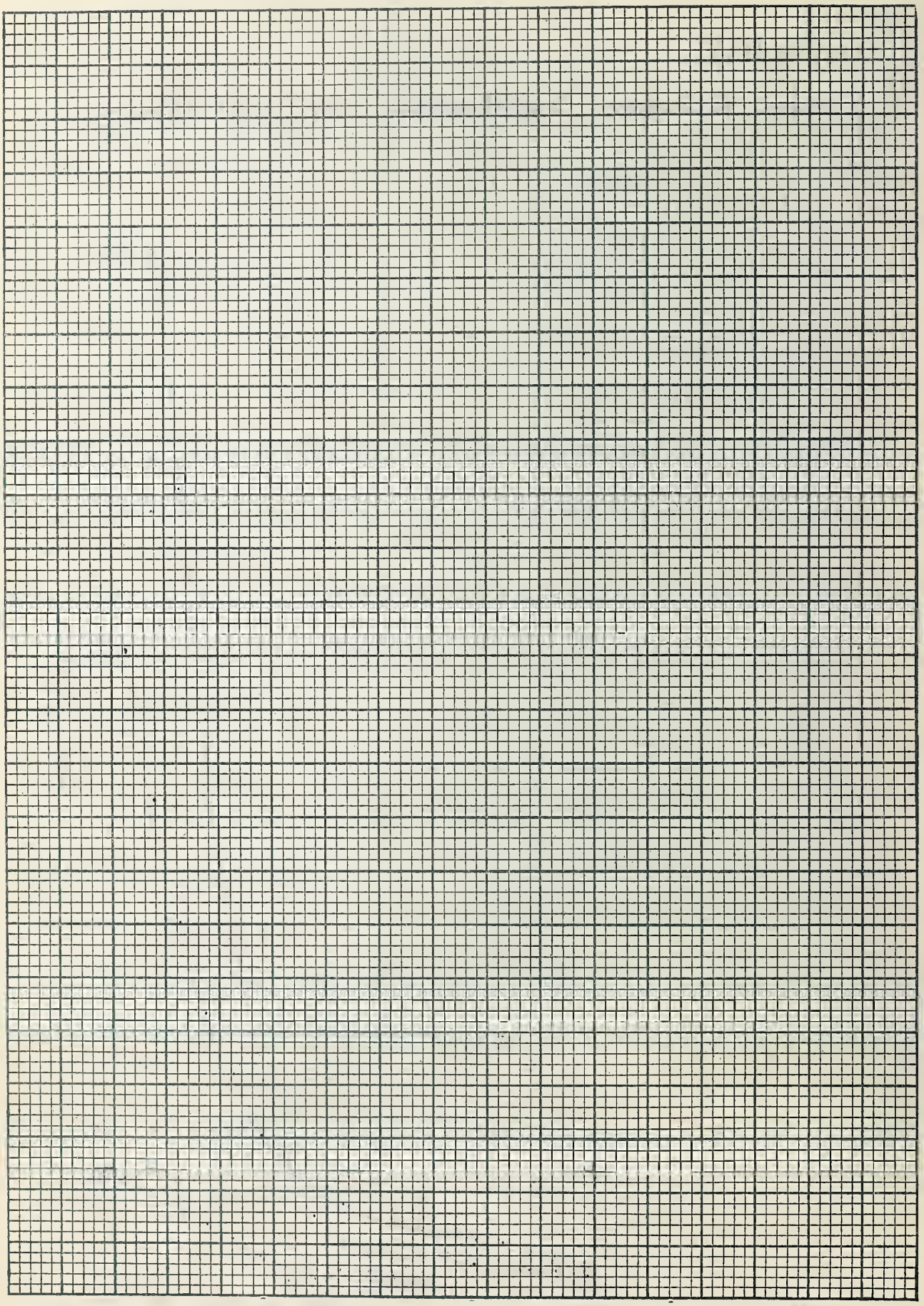


Figure (II)

Sodium Alizarin Sulphonate in Acid Solution





The indicator (14) used for the fluorine determination contains 0.17 mg. of sodium alizarin sulphonate and 0.87 mg. of zirconyl nitrate, $ZrO(NO_3)_2 \cdot 2H_2O$, per ml. Solutions containing this indicator were made up as follows:

Solution A 2 ml. of indicator + 2 ml. of 4.36N H_2SO_4 + 2 ml. of 2.95N HCl. Volume made up to 100 ml. with distilled water.

Solution B 1.8 ml. of indicator as above.

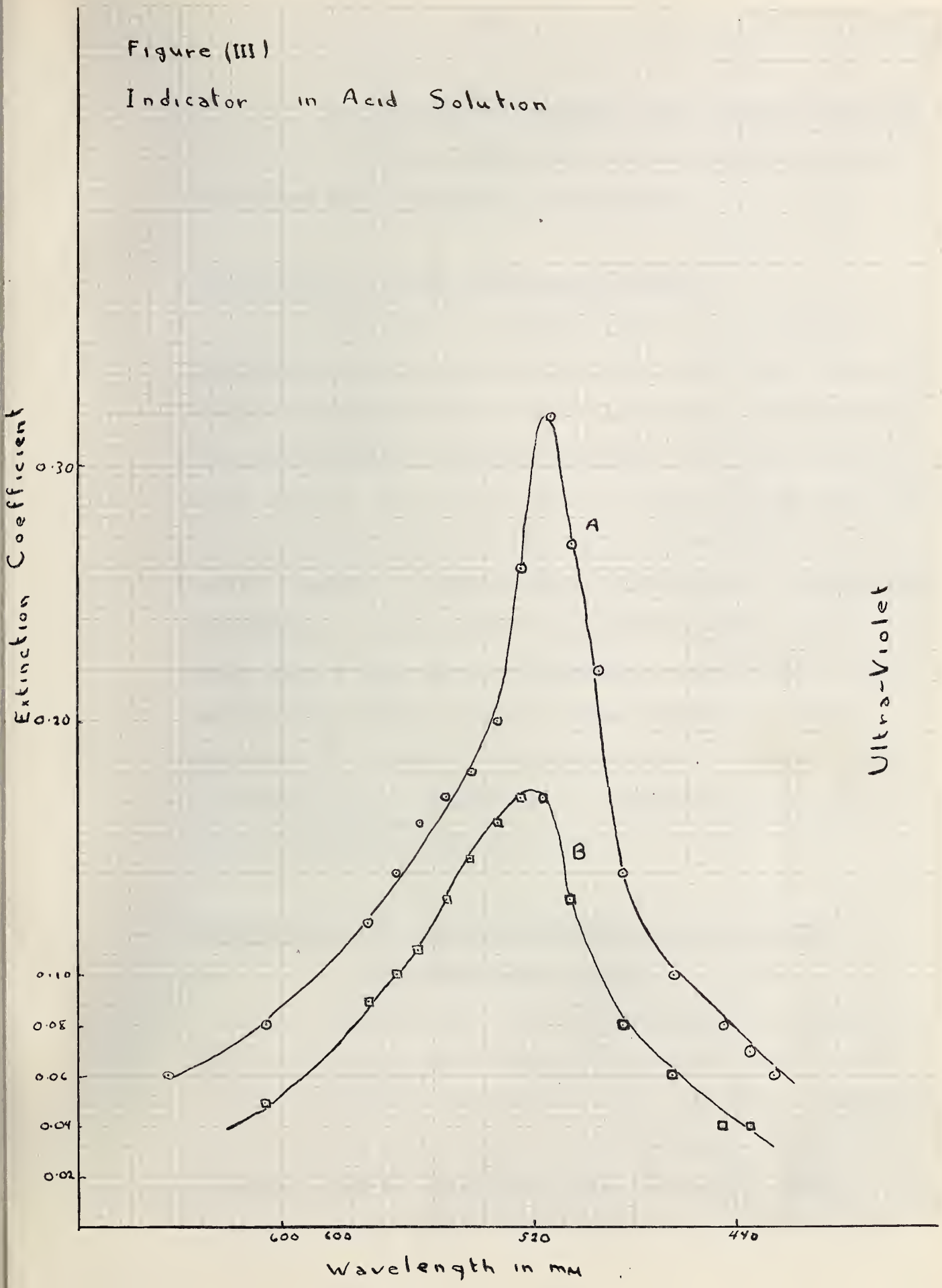
The extinction coefficients obtained at various wave lengths are tabulated in Table II and plotted in Figure (III)

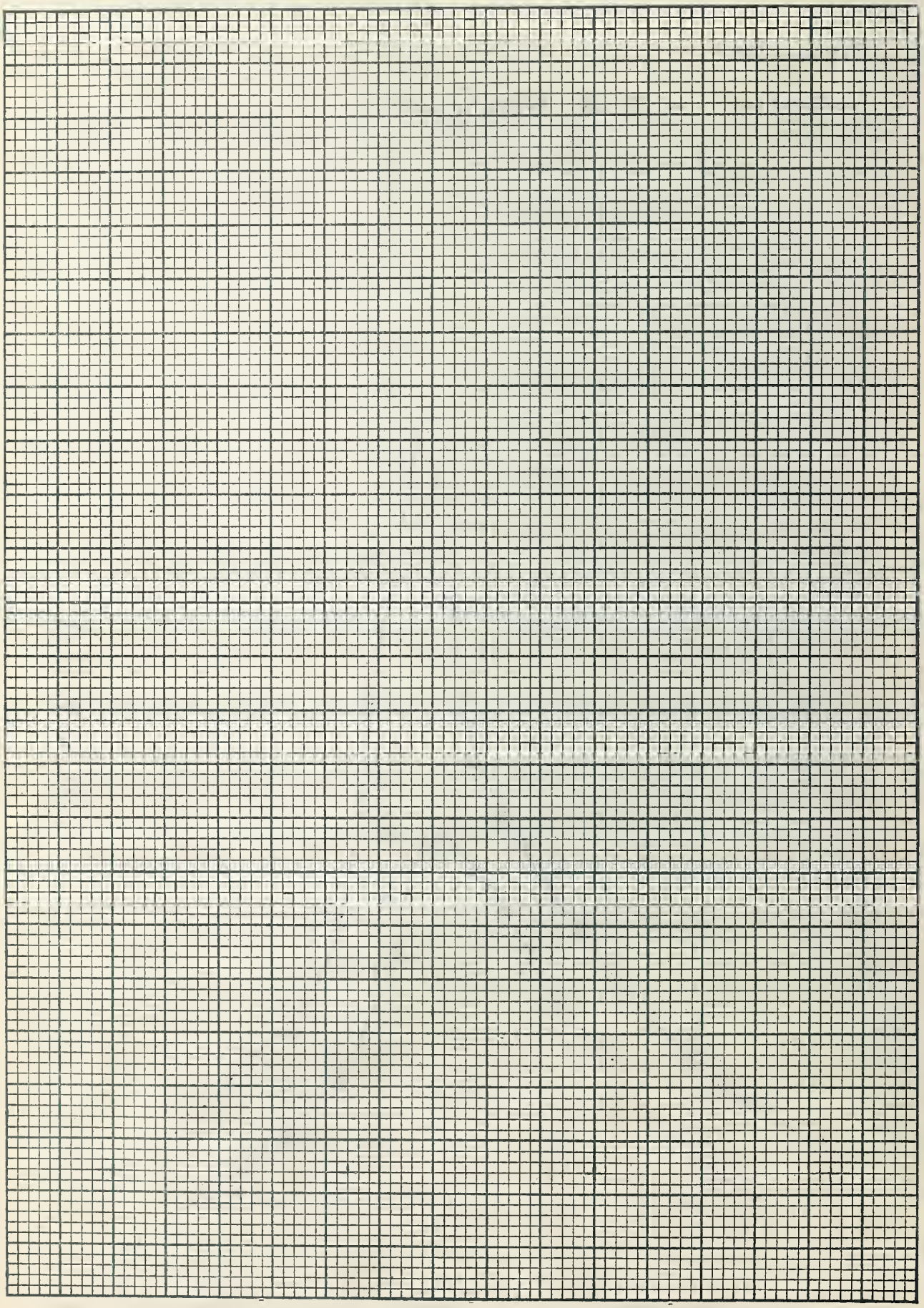
TABLE II

Wave length millimicrons	Extinction Coefficient A	B	Wave length millimicrons	Extinction Coefficient A	B
665	0.060		515	0.320	0.170
625	0.080	0.048	505	0.270	0.130
585	0.120	0.090	494	0.220	
575	0.140	0.10	485	0.140	0.080
565	0.160	0.11	465	0.100	0.060
555	0.170	0.13	445	0.080	0.040
545	0.180		435	0.070	0.040
535	0.200	0.16	425	0.060	0.030
525	0.260	0.17			

Figure (III)

Indicator in Acid Solution





In both cases above the lake precipitated out of solution to some extent and the concentrations are therefore not exactly as stated above.

Discussion of Spectrophotometric Curves

The curves of Figure I for the red form of sodium alizarin sulphonate indicate that light absorption is at a maximum at 515 - 520 millimicrons. The curves for the indicator solution (Fig.(III)) show maxima in the same region. The yellow form of sodium alizarin sulphonate has a maximum absorption in the ultra violet at a wave length somewhat less than 420 millimicrons. (Figure (II)) The curves for the yellow form indicate that the absorption due to this form is appreciable in the 515 - 520 millimicron range and that if the indicator solution contains an excess of the yellow dye as it does in the determination of fluorides the chances of Beer's Law being obeyed are rather remote.

Description and Use of the Lumetron Photoelectric Colorimeter Model 402-E

Results from the spectrophotometer indicate that a filter having maximum transmission near a wave length of 515 millimicrons will give the best calibration curve for the system under investigation. The filters supplied with the instrument have a spectral width (spectral range within which their transmission is 50%

of their peak transmission) of 20 - 30 millimicrons. The instrument used was manufactured by the Photovolt Corporation, New York. It is a null point instrument using two photocells, a slide wire and a galvanometer connected in a current bridge circuit. The balance of the circuit is indicated by the galvanometer. The light from a 100 candle power projection lamp, A, (Figure (IV)) is collimated by the optical system, L, to form a parallel beam. After passing through the monochromatic filter, J, the beam is split into two parts. One part of the beam passes through the solution in the sample holder, C, and strikes the measuring photocell, D. The other part of the beam is deflected by a mirror, E, to act on the balance photocell, F, which is mounted so that it can be rotated through an angle of 90°. When the active face of the cell is normal to the beam the cell receives the full light. When the cell is rotated through 90 degrees the edge of the active face is presented to the light and there is no action of the light on the cell. Thus the light striking the cell may be varied by use of the knobs Bc (coarse) and Bf (fine) to set the galvanometer to zero.

The galvanometer is a taut-wire, spot-light instrument with a coil resistance of 90 ohms and a sensitivity of 0.06 microamperes per division. Electrical

balancing of the bridge circuit is achieved by turning the adjustable contact arm of a slide wire which is provided with a calibrated dial. The slide wire consists of a large number of turns of low resistance wire. The slide wire scale is 9 inches long and calibrated in per centages. It can be read to a fraction of one per cent provided the sensitivity of the instrument produces a clearly detectable off-balance on the galvanometer for such small changes on the slide wire scale.

The photocells are the self generating barrier layer type. The light on the cells in normal operation is less than one foot-candle. At such low values of light the response of the photocells is completely linear. This linearity offers the advantage that straight line plots are obtained in calibrating the instrument.

A switch on the instrument allows it to be used as a direct reading instrument - the current from the measuring photocell alone is measured. Using this switch the lamp rheostat is adjusted to obtain a predetermined reading on the galvanometer.

Sample holders, supplied in different sizes with the apparatus have plane, parallel windows and are fused together so as to be resistant to any liquid which does not attack glass.

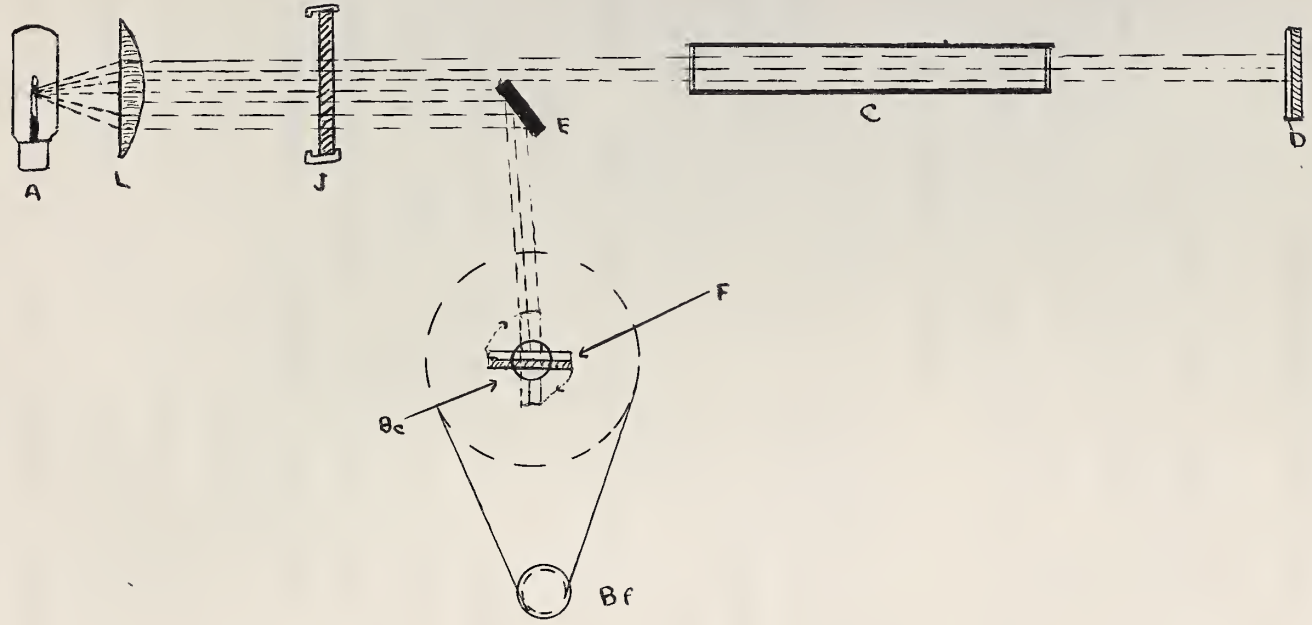


Figure (IV) : Optical System of the Lumetron Photoelectric Colorimeter

Calibration of the Photoelectric Colorimeter For the Determination of Fluoride

The colorimeter described above was used with filter 515. The sample holder was a cylindrical cell with a volume of 120 ml. and a light path of 150 mm. The procedure for determining data for the calibration curve is outlined below.

To 150 ml. of distilled water containing a known amount of added fluoride are added 3 ml. of 3 N hydrochloric and 3 ml. of 3 N sulphuric acids. This solution is placed in the sample holder of the photoelectric colorimeter, the slide wire set at 100% transmittancy and the galvanometer is brought to zero by adjusting the angles of the balance photocell (Bc and Bf Fig. (IV)). Then a measured amount of indicator is added and mixed. The percentage transmittancy of the solution is measured immediately after the addition of the indicator. The solution is then brought to the boil in a 300 c.c. Erlenmeyer flask and allowed to stand for four hours. The percentage transmittancy is again determined. The optical density

$$\left(\log_{10} \frac{I_o}{I} = \log_{10} 100 - \log_{10} \%\ \text{transmittancy}\right)$$

is calculated for each reading and the

decrease is determined. A sample of distilled water with acids added as above and the same amount of indicator, but with no added fluoride is treated in the same manner. The decrease in optical density for this solution gives the decrease due to the acids added. The decrease due to fluorides is obtained by subtraction. Thus the calibration curve will show decrease in optical density due to the fading action of fluoride for various concentrations and will be independent of the exact concentration of acids used.

Transmittancy measurements were made on standards using 2.7 ml. of indicator in one case and 3 ml. in the other in order to see if the amount of indicator influenced the curve.

The data are shown in Table III.

TABLE III

	Optical density decrease due to F^-	
*p.p.m.F^-	2.7 ml. of indicator	3.0 ml. of indicator
0.2	(3) 0.026	(3) 0.031
0.4	(4) 0.055	(2) 0.055
0.5	(2) 0.058	
0.7	(3) 0.073	(3) 0.073
0.8	(2) 0.083	
1.0		(2) 0.96
1.1	(3) 0.110	
1.2		(2) 0.107
1.3	(2) 0.128	
1.4	(4) 0.142	(3) 0.112
1.5	(2) 0.156	(3) 0.116

* p.p.m. = parts per million.

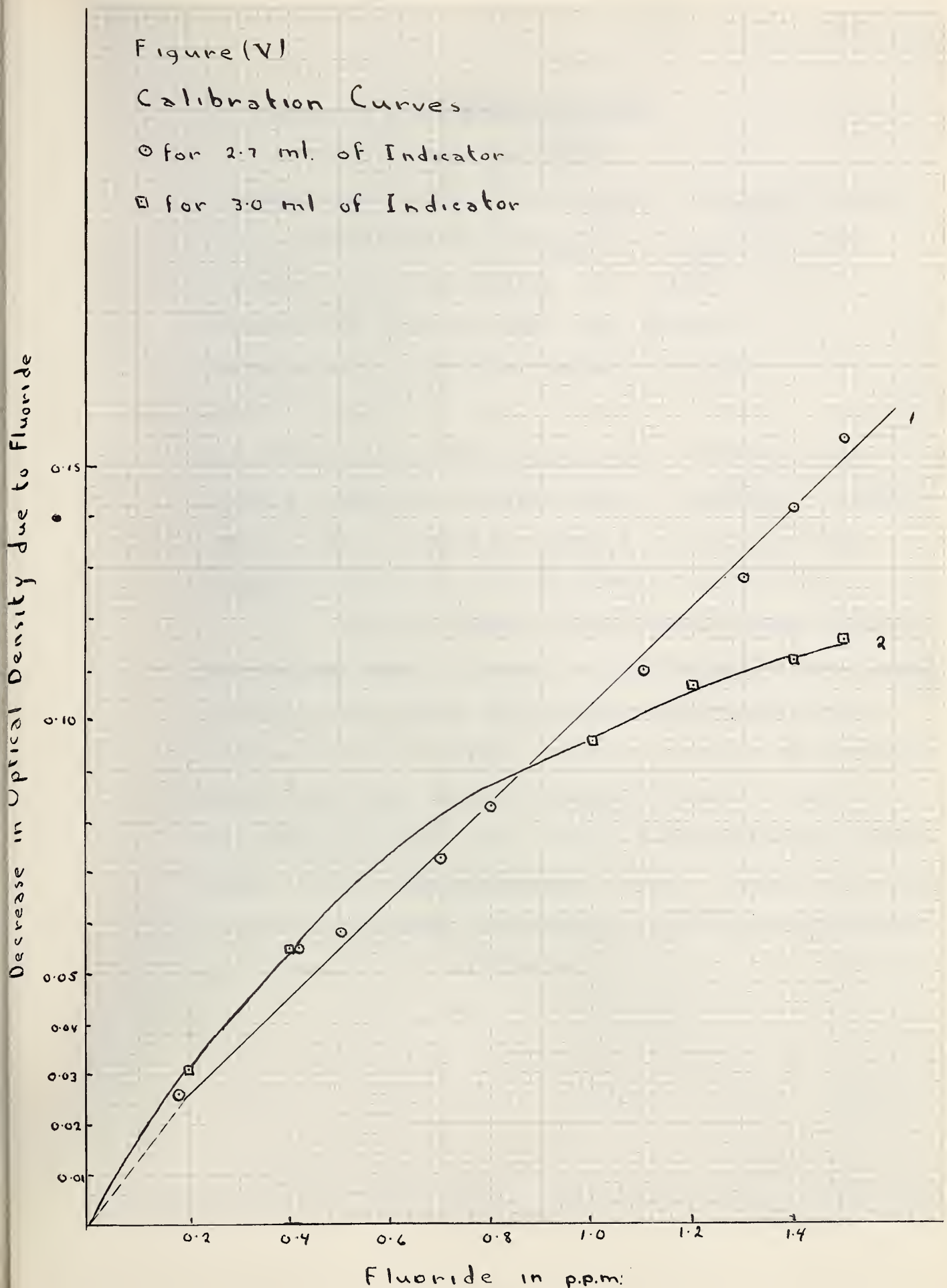
The numbers in brackets in Table III indicate the number of determinations used to calculate the average. The optical densities shown in Table III were calculated as follows using as an example the determination involving 0.2 p.p.m. of fluoride and 3.0 ml. of indicator.

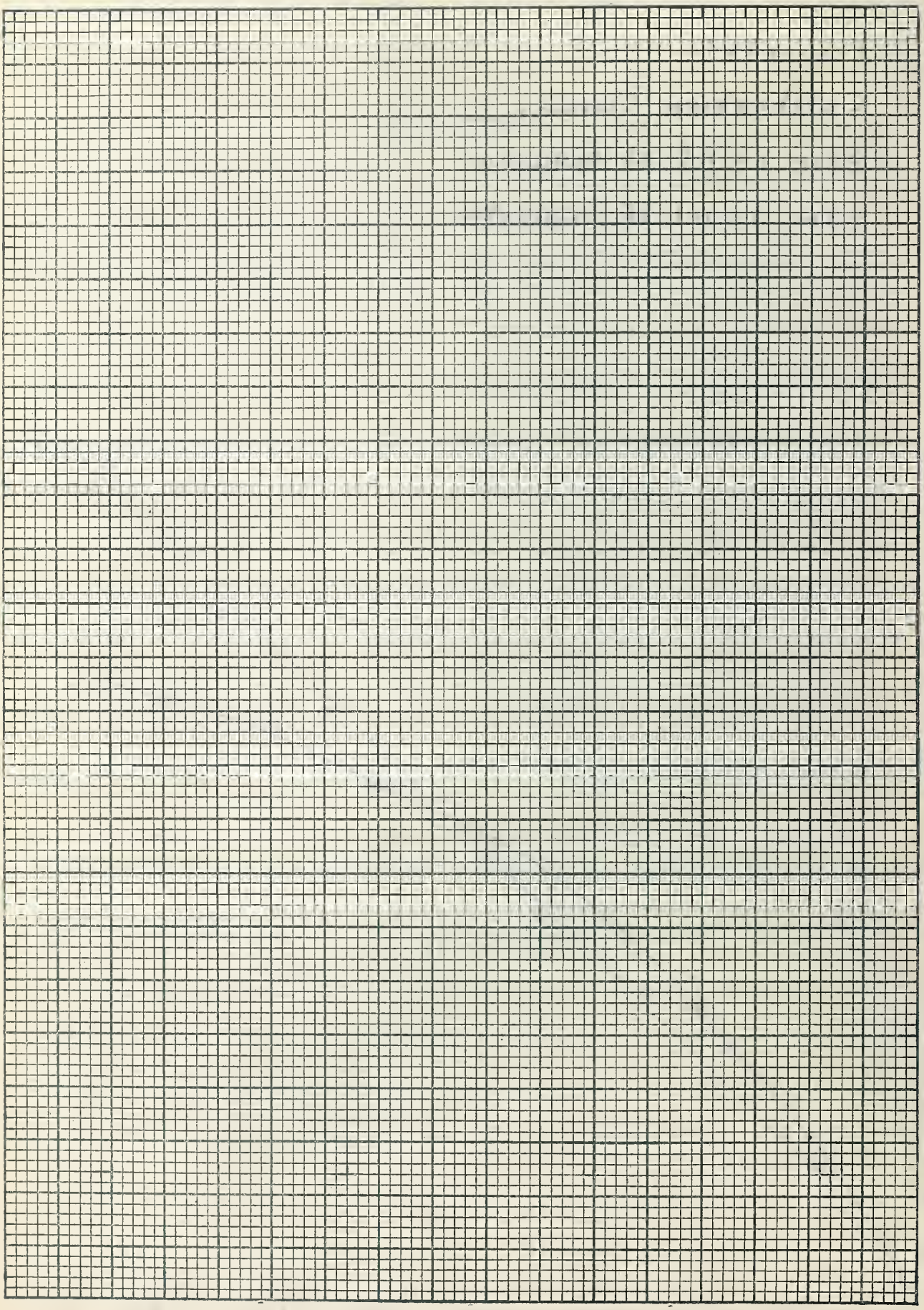
Figure (V)

Calibration Curves

⊙ for 2·7 ml. of Indicator

⊡ for 3·0 ml of Indicator





Original % transmittancy 22.7

Final % transmittancy 33.8

Original optical density = $\log_{10}100 - \log_{10}22.7 = 0.644$

Final optical density = $\log_{10}100 - \log_{10}33.8 = 0.471$

Decrease in optical density = 0.173

Decrease due to acids added = 0.142

Decrease due to fluorides present = 0.031

The decrease in optical density due to fluorides is plotted against the concentration of fluorides in parts per million in Figure V. Curve 1 is for 2.7 ml. of indicator and curve 2 is for 3 ml. of indicator.

It is necessary to make two determinations of the optical density and use the difference, since a small change in the amount of indicator added causes large changes in this density. This was shown by the erratic results obtained when an attempt was made to calibrate the instrument with only a final % transmittancy measurement. If only one measurement is made the indicator must be exactly the same concentration for each determination as it was for the calibration.

Discussion of the Calibration Curves

The curve obtained by using 2.7 ml. of indicator is a straight line for a large part of the range and has a steeper slope than the curve for 3 ml. of indicator. Therefore it is better to use the smaller volume. Curve 1, Figure (V) indicates that the optical properties of the system change at approximately 0.2 parts of fluoride per million parts of water. Since the system does not appear to conform to Beer's Law below 0.2 p.p.m. fluoride it was decided to examine the optical properties of the yellow form of sodium alizarin sulphonate and the red lake at wave lengths of 515 and 420 millimicrons.

As noted under the section on "Theory of Colorimetry", solutions conforming to Beer's Law show a constant molecular extinction coefficient at all dilutions and thicknesses for a given wave length. Another proof of the validity of this law lies in a straight line plot for $\log_{10} I/I_o$ (log T) or $\log I_o/I$ (optical density) against concentration.

Therefore % transmittancy measurements were made with the photoelectric colorimeter using wave lengths of 515 and 420 millimicrons. The data are shown in Table IV and curves are plotted in Figures (VI) sodium alizarin sulphonate using filter 420; (VII) sodium alizarin

sulphonate using filter 515; (VIII) indicator solution using filters 515 and 420.

TABLE IV

Sodium Alizarin Sulphonate (yellow form)

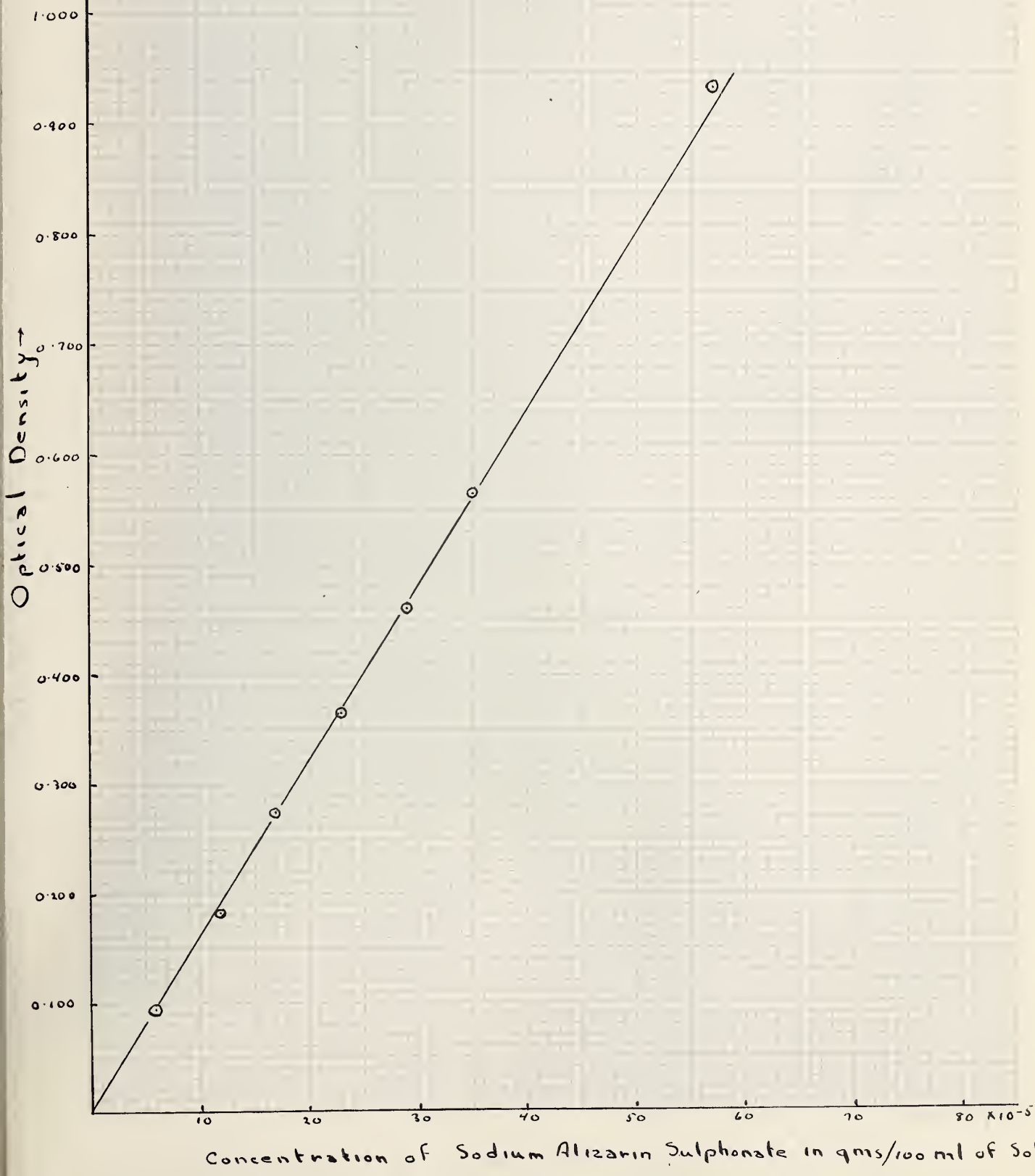
Concentration in gms./100 ml.	Filter 420		Filter 515	
	Log. % Transmittancy	Optical Density	Log. % Transmittancy	Optical Density
6×10^{-5}	1.909	0.091	1.998	0.002
12×10^{-5}	1.818	0.182	1.997	0.003
17×10^{-5}	1.728	0.272	1.996	0.004
23×10^{-5}	1.634	0.366	1.991	0.009
29×10^{-5}	1.540	0.460	1.987	0.013
35×10^{-5}	1.436	0.564	1.984	0.016
57×10^{-5}	1.061	0.939		
110×10^{-5}	0.176	1.824		
Indicator Solution*				
11.3×10^{-5}	1.902	0.098	1.806	0.194
16.9×10^{-5}	1.848	0.152	1.712	0.288
22.6×10^{-5}	1.804	0.196	1.609	0.391
28.2×10^{-5}	1.758	0.242	1.515	0.485
34×10^{-5}	1.706	0.294	1.412	0.588

* The concentration of the indicator is expressed as grams of sodium alizarin sulphonate contained per 100 ml. of solution.

Figure (VII)

Sodium Alizarin Sulphonate in Acid Solution

Filter 420



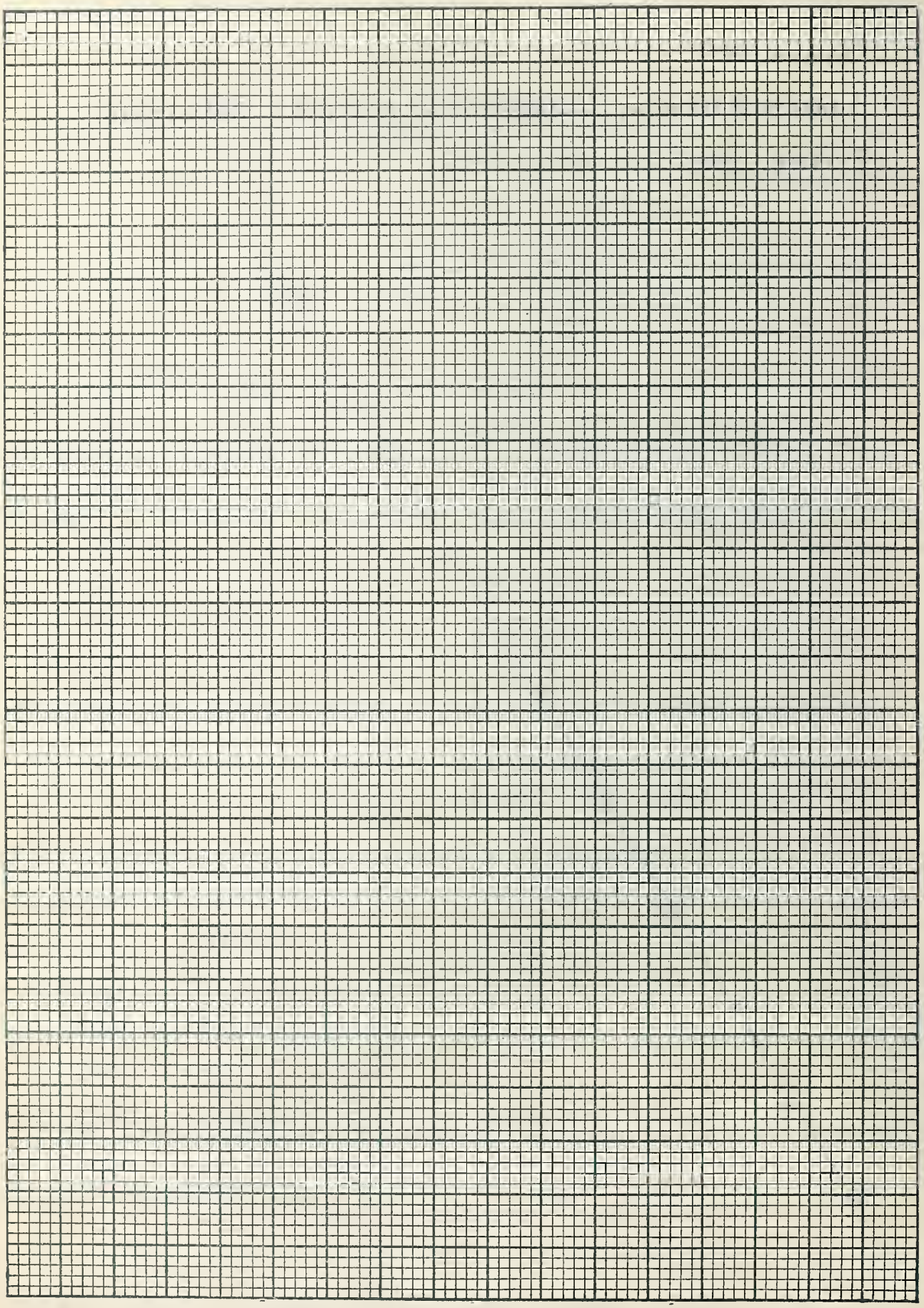
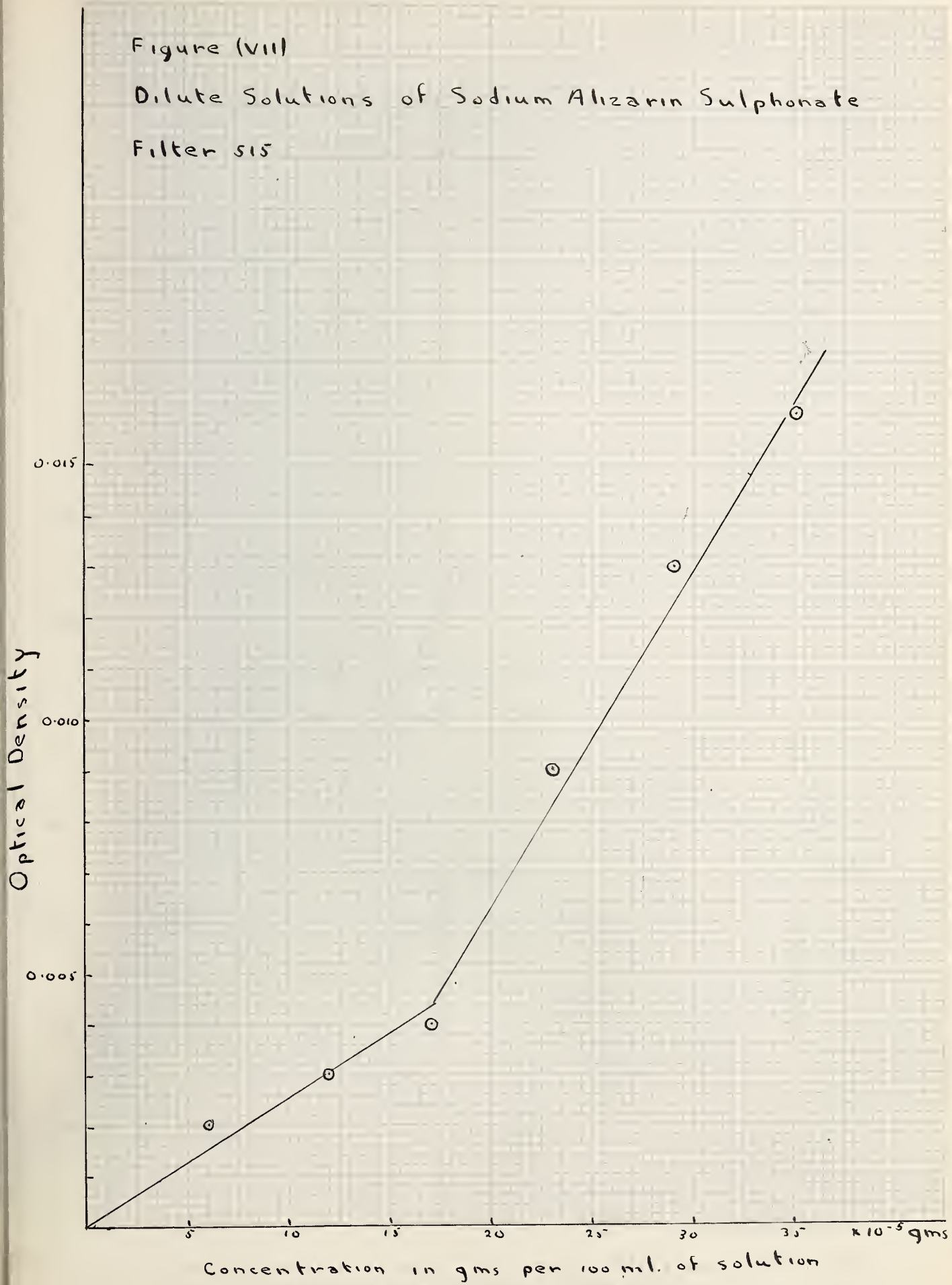


Figure (VII)

Dilute Solutions of Sodium Alizarin Sulphonate

Filter 515



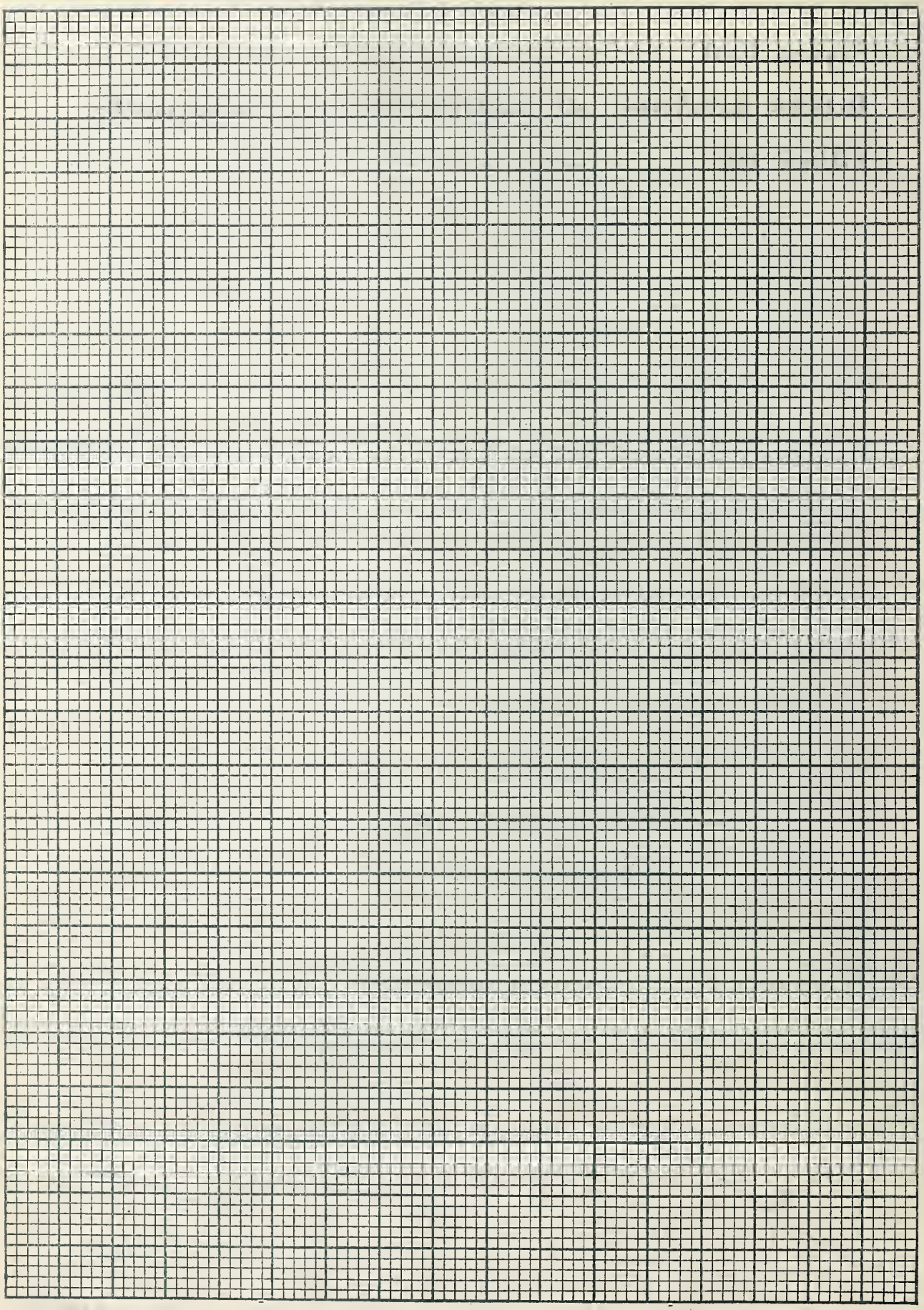
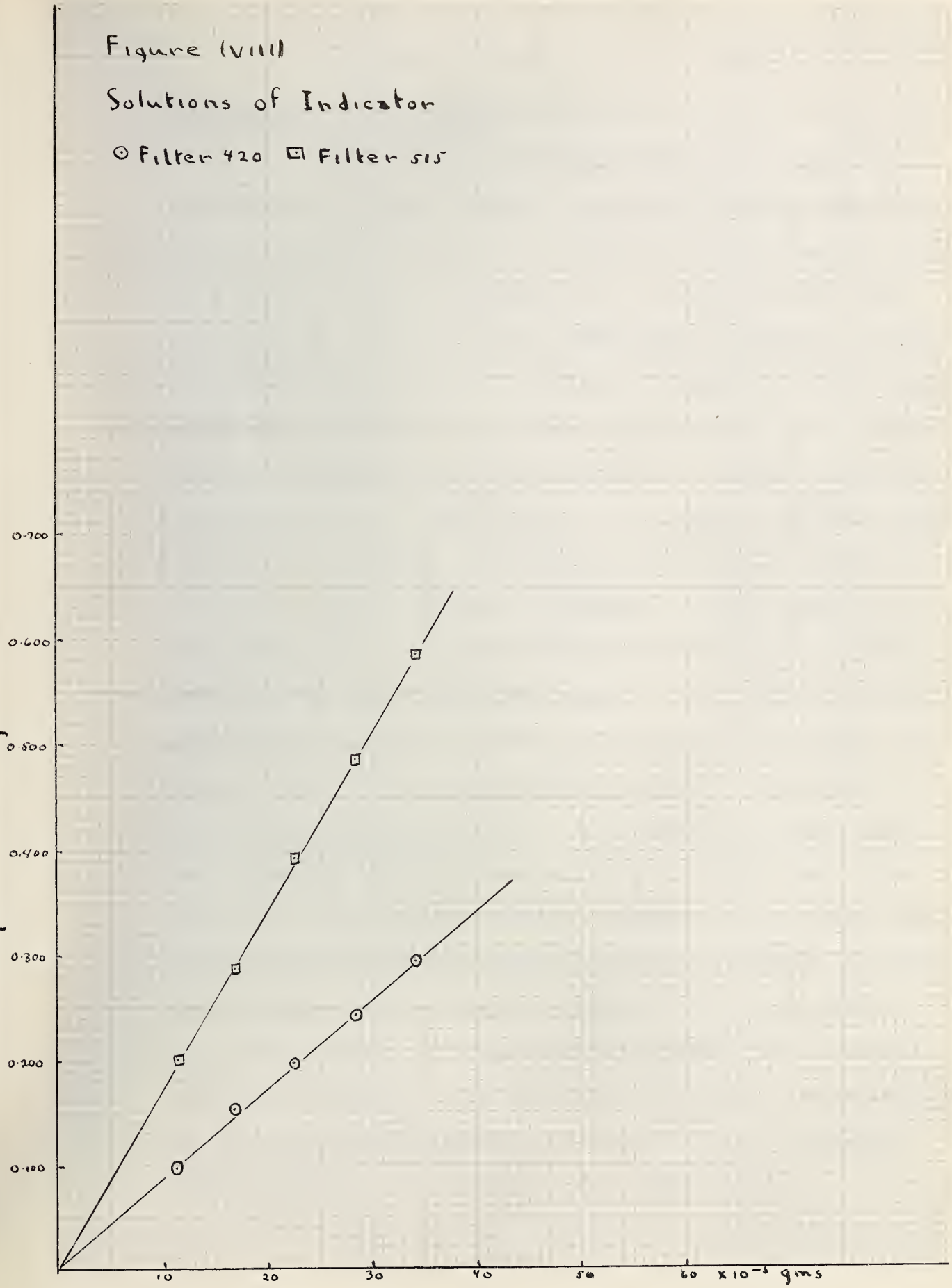


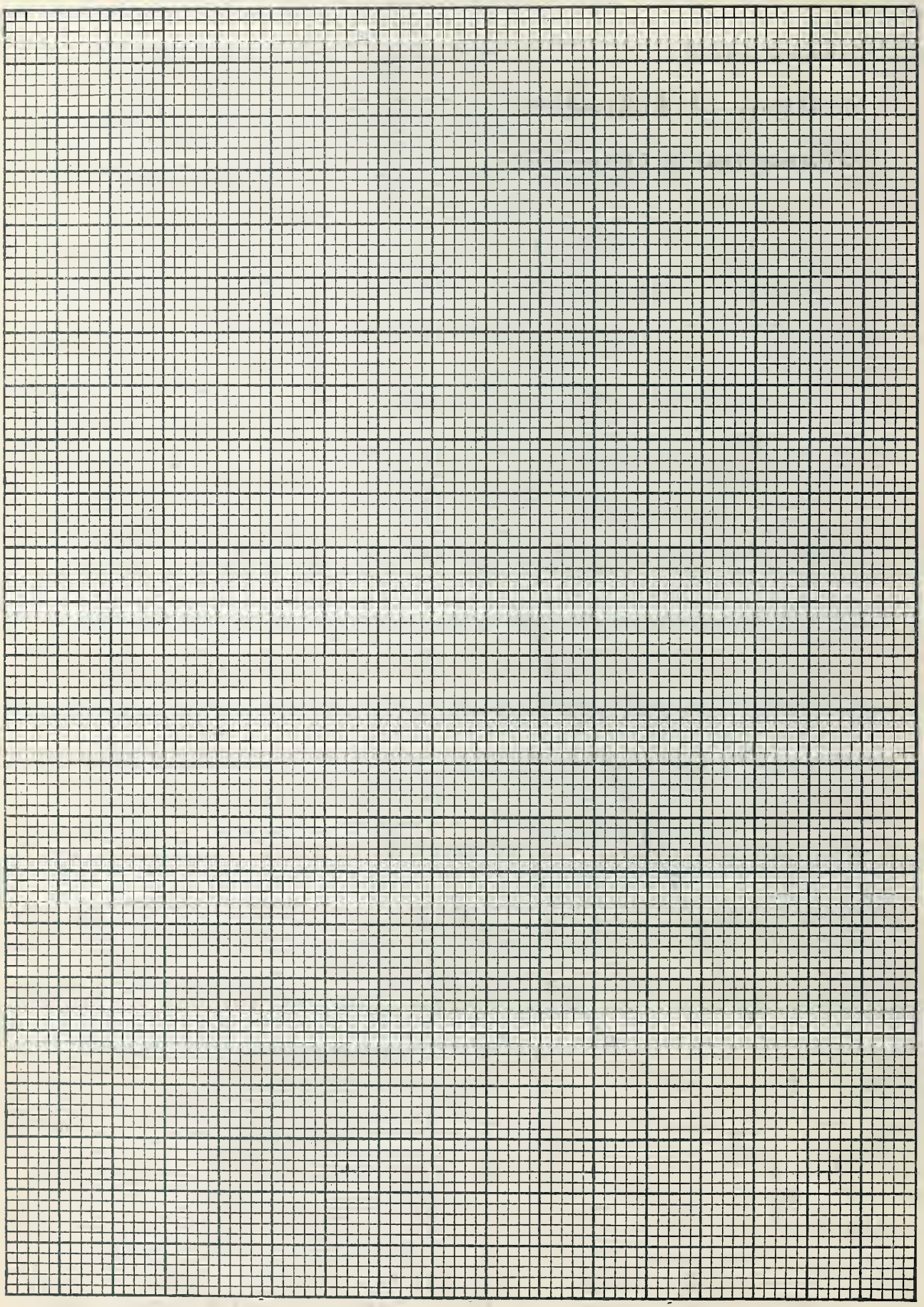
Figure (VIII)

Solutions of Indicator

⊙ Filter 420 ⊡ Filter 515



Concentration as gms of Sodium Alizarin Sulphonate contained per 100 ml. of solution.



Discussion of the Curves

The curve, Figure (VII) using filter 515 for solutions of sodium alizarin sulphonate in acid solution shows that Beer's Law is not valid for low concentrations. In using 1.8 ml. of indicator per 100 ml. of water (14) the solution contains 30.6 X 10^{-5} grams of sodium alizarin sulphonate in 100 ml. of solution. Fluorides in the water cause the release of the sulphonate from the lake. The curve in Figure (VII) changes slope at a concentration of approximately 17 X 10^{-5} grams of the yellow sulphonate per 100 ml. of solution. A property of bicolored substances (of which sodium alizarin sulphonate is one) noted by Dehn (18) may be an explanation. The statement is "at sufficiently great dilution, many, if not all, bicolored substances whether in acidic or in alkaline solutions are of the same tint". Since sodium alizarin sulphonate is a bicolored acid-base indicator this dilution effect may be an explanation for the non-validity of Beer's Law. The change in slope for the optical density vs. concentration curve (Figure (VII)) for sodium alizarin sulphonate probably corresponds to the change in slope of the calibration curve for 2.7 ml. of indicator (Figure (V)). Beer's Law is seen to be valid for the indicator solution at wave lengths of 515 and 420 millimicrons (Figure (VIII)) and also for sodium alizarin sulphonate at 420 millimicrons.

As a test of the method the fluoride content of some natural Alberta waters were determined and results were compared with those obtained by use of the visual method according to Walker and Finlay (14). The samples were treated in the same manner as the standards used in obtaining the calibration curve. A comparison of results obtained by this and the visual method is shown in Table IV. Results from the two methods are seen to be in agreement.

TABLE IV

Sample	p.p.m.F^- Visual	p.p.m.F^- Photo-electric colorimeter
#1	0.3	0.15
#2	0	0
#3	0	0
#4	0	0
#5	0.1	0
#6	0.1	0.1
#1 + 0.7 p.p.m.F^-	0.9	0.85
#2 + 0.7 p.p.m.F^-	0.6	0.6
#3 + 0.7 p.p.m.F^-	0.5	0.5
#6 + 0.4 p.p.m.F^-	0.5	0.5
#6 + 0.7 p.p.m.F^-	0.8	0.7
#6 + 1.2 p.p.m.F^-	1.0	1.0

Conclusions

In this research an adaptation of the visual method for the determination of fluorides has been tested for the Lumetron Photoelectric Colorimeter. Results have been found to be in agreement with those obtained by the standard Sanchis visual method. The method shows possibilities of determining fluoride concentration to the second decimal in parts per million if this accuracy is desired.

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SECTION II

THE MICRODETERMINATION OF IODINE IN CEREAL GRAINS OF ALBERTA

The Microdetermination of Iodine in Cereal Grains of Alberta

Experiments in animal feeding are now being conducted in the Agricultural Department of the University of Alberta. Cereal grains from various parts of Alberta are being used as feed for domestic animals. In order to complete the information gained from a study such as this, it is necessary to know the composition of the feed with respect to metabolically important elements. Thus it was decided that the iodine content of the grains used should be known. The research reported in this section of the thesis is for this purpose. It was financed by the Committee on Agricultural Research and was a part of Agricultural Research Project #4.

Iodine is known to play an essential role in human and animal metabolism. Iodine in the diet is necessary for the proper functioning of the thyroid gland, which gland has a far reaching effect on metabolism, reproduction, general health and resistance to bacterial infections (19). Foodstuffs and water upon which animals are maintained contain small and varying amounts of iodine, the variation depending on the kind of food and the source. There should be an inverse

relationship between the amounts of iodine in the food of a district and the incidence of the symptoms of goitre.

Work has been done on the iodine content of Alberta waters but the correlation has not been found to be as close as in other countries (18). Perhaps the best method of attack would be the mapping of a symptom of iodine deficiency in a domestic animal in conjunction with determinations of the iodine content of the feed (16).

Review of the Methods for Microdetermination of Iodine

The greatest difficulty in the determination is the destruction, without the loss of iodine, of the large amounts of organic matter. The very minute amounts of this element in some plant materials necessitate the use of large samples and thus large amounts of organic material. Alkali fusion methods used by early workers are applicable only to small samples and hence to materials of relatively high iodine content. These methods also use large amounts of reagents and there is therefore a danger of contamination. Remington used a low temperature ignition procedure and showed

good recoveries of iodine added as thyroid extract (12). Large losses of iodine added as iodine compounds with this method were reported by Reith (15). The method of Pfeiffer (14) is inapplicable to large samples and requires costly and fragile apparatus. A silica tube furnace was developed by McClendon (11) and adopted with modifications by other workers. The method consists of ashing the sample in a semi-enclosed system and absorbing the gases evolved. The absorption system must be large and the time saved in the ashing is lost in evaporation and manipulation of the large volumes of absorption solution. There is also the danger of loss of iodine due to reaction with nitrites (formed during the combustion by the fixation of atmospheric nitrogen). A proposal by Karns (6), of a slower, more easily controlled combustion in a specially constructed flask allows the use of much smaller volumes of absorption solutions.

The research to be reported in this section of the thesis deals with two of the combustion methods - the low temperature ignition of Remington (12) and the simplified Karns method of Von Kolnitz and Remington (17).

Methods of Analysis to be Tested

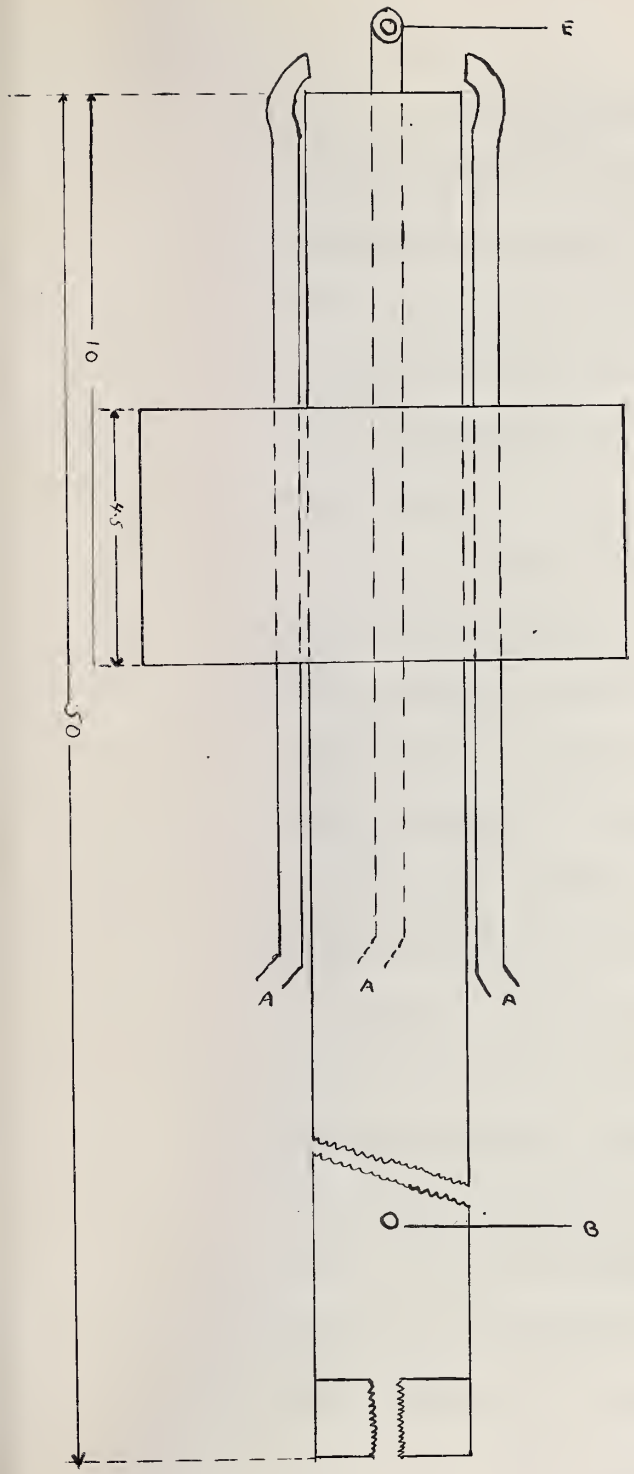
Earlier work was done on the simplified Karns method of Von Kolnitz and Remington as outlined below.

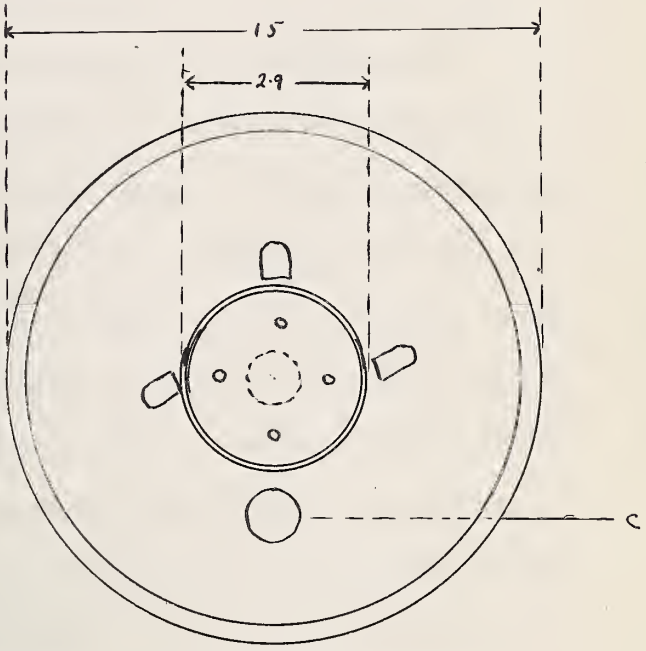
The sample is burned in a stream of oxygen in a torch, the details and specifications of which are given in the accompanying diagram (Figure I). The torch is constructed of brass and combines a screw feed device, oxygen inlets, an outlet tube to the absorption train, and a cup providing a water seal for a wide-mouthed Erlenmeyer-type flask inverted over the burning sample.

The sample is packed into a Visking sausage casing and placed in the tube of the torch with the end of the casing (beyond the knot) engaged in the four platinum points on the screw feed device. The cartridge is caused to rotate as it is fed into the region of combustion. The oxygen tubes are offset to give the gaseous stream a whirling motion. The outlet tube is of glass as the products of combustion are corrosive. The exposed brass parts are coated with nitro-cellulose lacquer to prevent copper from appearing in the washings. The feed tube has a small hole near the bottom to guard against overflow of the solution in the cup due to sudden changes in pressure.

Figure 1

Torch for Microdetermination of Iodine





Detail

A - Oxygen feed tubes

B - Air Vent - 3 mm. diam.

C - Hole for outlet tube - 9 mm. diam.

E - Fine jet - plug with 1mm. hole

Dimensions are in cm.

The absorption train consists of two or three Milligan wash bottles in series. The electrical precipitator for dust particles is dispensed with as being inconvenient in operation and of little value in the analysis. Two pellets of sodium hydroxide are added to the first wash bottle and one to the succeeding wash bottles.

After the combustion the flask is washed with the solution from the cup and added to the solutions and washings from the wash bottles. The combined solutions are then evaporated to a small volume and transferred to a platinum dish where the evaporation is continued to dryness. A pellet of sodium hydroxide is added and the contents of the dish fused by waving over a flame. This material is taken up with water, the solution is filtered and the filtrate neutralized with sulfuric acid. Then a saturated solution of potassium or sodium carbonate is added till the solution is basic and it is evaporated to dryness. The solid salts are ground in a mortar and extracted five times with 95% ethyl alcohol (total volume should be approximately 150 ml.) The alcoholic solution is then evaporated to dryness. If the residue is colored it is heated in a muffle furnace at 400°C to oxidize organic matter which

may be present. Water is added to the residue. Then a few milligrams of sodium azide (to destroy nitrites) a few crystals of sodium sulfite (to reduce iodate to iodide) and one ml. of 85% phosphoric acid is added. The solution is boiled to remove excess sulfur dioxide and filtered into a graduated separatory funnel. The volume is made up to the 25 ml. mark and 2.5 ml. of C.P. carbon tetrachloride are added. Then a few crystals of sodium nitrite are added and the funnel stoppered and shaken 100 times to ensure equilibrium between the two layers. The solution of iodine in carbon tetrachloride is drawn off the bottom into a Dubosq colorimeter cup. A cork with a glass rod reaching into the solution is used to prevent evaporation. This introduction of a fresh surface helps collect any dispersed water droplets and the turbidity disappears. The cork may be removed and the glass rod wiped clean. The color of the solution is then compared with that produced by a standard solution of iodine.

The second method of ashing investigated is the low temperature ashing procedure suggested by McClendon and Remington (12). The original method is outlined below.

A sample of cereal grain (which gives an acid ash) is moistened with 50 cc. of 2% calcium lactate solution followed by 50 cc. of 2% sodium carbonate solution. The sample is dried, coarsely ground and heated in an evaporating dish over a small flame till the vegetable matter begins to smolder. The sample is allowed to smolder with no flame till reduced to a black char. Then the dish is placed in a muffle furnace at a temperature not exceeding 450°C until the ash is light grey in color. The ash is then ground with water to extract soluble salts until reduced to an impalpable powder. The solution is decanted and freed of ash and then evaporated to a small volume. The contents are transferred to an evaporating dish and dried in an electric oven. They are then placed in a nickel boat and heated in a pyrex combustion tube, till traces of organic matter which may have escaped the first combustion are destroyed. Air is allowed to pass over the heated salts and exit gases are led to a test tube of sodium hydroxide solution. The ash from this combustion is dissolved in the sodium hydroxide solution. A mixture of 90 parts of syrupy phosphoric acid and 10 parts of sulfurous acid solution (0.1N or stronger) are added, drop by drop until effervescence ceases. The solution

is boiled to expel sulfur dioxide. It is tested with indicator paper (an alcoholic solution of brom phenol blue evaporated on ash-free filter paper). If the color does not change from blue to yellow, sulfuric acid is added drop by drop until it does. This solution is then put through the same procedure as the solution of the alcohol residue in the torch method.

Theory of the Methods

The minute amounts of iodine in cereal grains necessitates the use of large samples and consequently large amounts of reagents. Because of the large number of interfering compounds which must be destroyed before the iodine can be determined and the minute amounts to be determined, careful manipulation and control of conditions are of prime importance.

The absorption solution from the torch method contains the iodine as iodide and iodate.

Organic iodine compound + $O_2 \rightarrow I_2 + HI + HIO_3$

$3\ I_2 + 6\ KOH \longrightarrow 5\ KI + KIO_3$

In cases requiring reduction of the amount of soluble salts by an alcohol extraction the iodate must be reduced to the iodide, since iodate is not soluble in alcohol.

This is accomplished by von Kolnitz and Remington (17) by addition of sodium sulfite with phosphoric acid.

(1) $3\ SO_3^{=} + IO_3^{-} \rightarrow 3\ SO_4^{=} + I^{-}$

In the low temperature ashing procedure phosphoric and sulphurous acids are added to the residue from the second combustion. Thus any heavy metal ions may be removed (insoluble phosphates) by filtration early in the procedure before they are able to interfere.

(2) $2\ Fe^{+++} + 2\ I^{-} \rightarrow 2\ Fe^{++} + I_2$

(3) $2\ Cu^{++} + 4\ I^{-} \rightarrow 2\ CuI + I_2$

Phosphoric acid transforms iron into a complex so that it no longer reacts with iodide.

The careful control of conditions is shown to be necessary to prevent loss of iodine by a review of the oxidation-reduction theory of iodine, iodide and iodate interaction (8).

In the reaction

$$I_2 + 2\ e \rightleftharpoons 2\ I^{-}$$

the iodide exerts a reducing action on strong oxidizing agents with the production of an equivalent amount of free iodine. Below a pH of 8 the oxidation potential of the iodine-iodide system is independent of hydrogen

ion concentration; but above this, iodine reacts with hydroxyl ions to form hypoiodite and iodide. The hypoiodite is transformed into iodate.

(4) $I_2 + 2\ OH^- \longrightarrow IO^- + I^- + H_2O$

$3\ IO^- \longrightarrow 2\ I^- + IO_3^-$

In acid solution the following reactions may occur:

(5) $2\ MnO_4^- + 10\ I^- + 16\ H^+ \longrightarrow 2\ Mn^{++} + 5I_2 + 8\ H_2O$

(6) $BrO_3^- + 6\ I^- + 6\ H^+ \longrightarrow Br^- + 3\ I_2 + 3\ H_2O$

(7) $IO_3^- + 5\ I^- + 6\ H^+ \longrightarrow 3\ I_2 + 3\ H_2O$

(8) $4\ I^- + 4\ H^+ + O_2 \longrightarrow 2\ I_2 + 2\ H_2O$

(9) $2\ NO_2^- + 2\ I^- + 4\ H^+ \longrightarrow 2\ NO + I_2 + 2\ H_2O$

Reaction (8) is very slow in neutral solution but the rate is greatly increased by an increase in hydrogen ion concentration, by direct sunlight, by a reaction between the iodide and some reducible substance (an induced reaction), and by the presence of certain metallic ions as cupric copper which act as catalysts. Minute traces of nitrites interfere as shown in reaction (9). Nitric oxide reacts with atmospheric oxygen to form higher oxides which again react with iodide. These considerations indicate that interfering anions, such as nitrites, and ions exerting a catalytic effect on reaction (8) must be removed before acidification. The iodine should be in one form, either iodate or iodide, but not both when acidified or a loss will result from reaction (7).

Procedure in the Colorimetric Step

The reaction shown below was used to form iodine from iodides.

$$2\ NO_2^- + 2\ I^- + 4\ H^+ \longrightarrow 2\ NO + I_2 + 2\ H_2O$$

As noted above the iodine is dissolved in carbon tetrachloride and the amount of iodine is determined by transmittancy measurements. In the first part of the work, a Dubosq colorimeter was used to compare the color due to iodine from a sample with the color of a standard solution of iodine. Later the Lumetron Photoelectric colorimeter, as described in the Fluorine section of this thesis was used.

A spectrophotometric curve was obtained for two concentrations of iodine in carbon tetrachloride. In Solution 1 the concentration of the iodine was 48 γ * per ml. and in Solution 2 it was 1800 γ per ml. The data are shown in Table I and plotted in Figure 2. The curves indicate that the solution absorbs maximum light at a wavelength of 520 millimicrons. The similar shapes of the curves and the fact that the extinction coefficient is proportional to the concentration points to the validity of Beer's Law for the system.

* 1 gamma (γ) = 1×10^{-6} grams.

TABLE I

Extinction Coefficients for Iodine Solutions

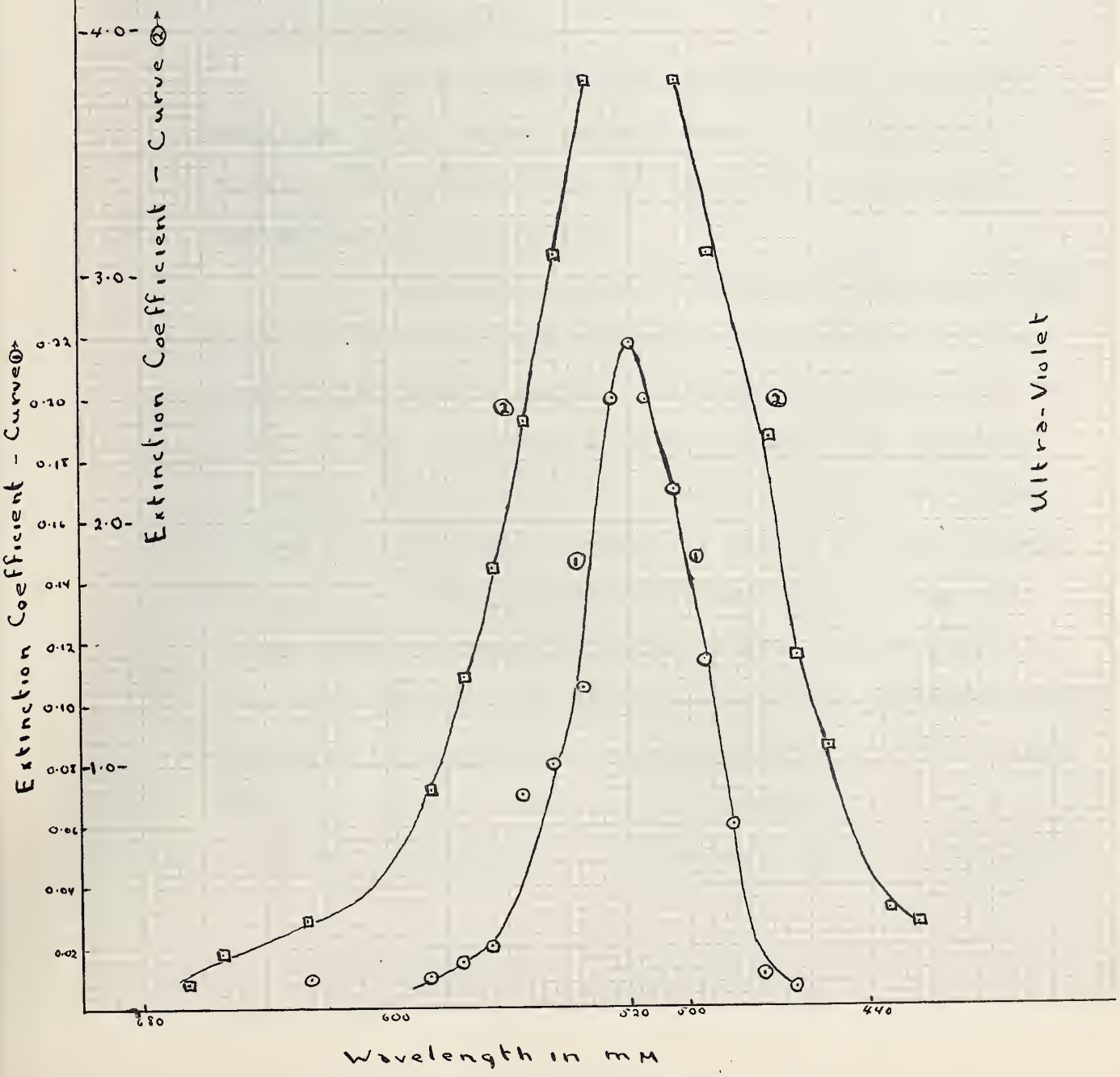
Wave length in millimicrons	Extinction Coefficient Solution 1	Solution 2
665		0.10
655		0.18
625	0.01	0.37
585	0.01	0.91
575	0.015	1.36
565	0.02	1.82
555	0.07	2.42
545	0.08	3.10
535	0.105	3.80
525	0.200	over 4.0
520	0.219	
515	0.200	over 4.0
505	0.17	3.80
495	0.115	3.10
485	0.06	2.35
475	0.01	1.80
465	0.00-0.01	1.46
455		1.08
445		0.64
435		0.41
425		0.35

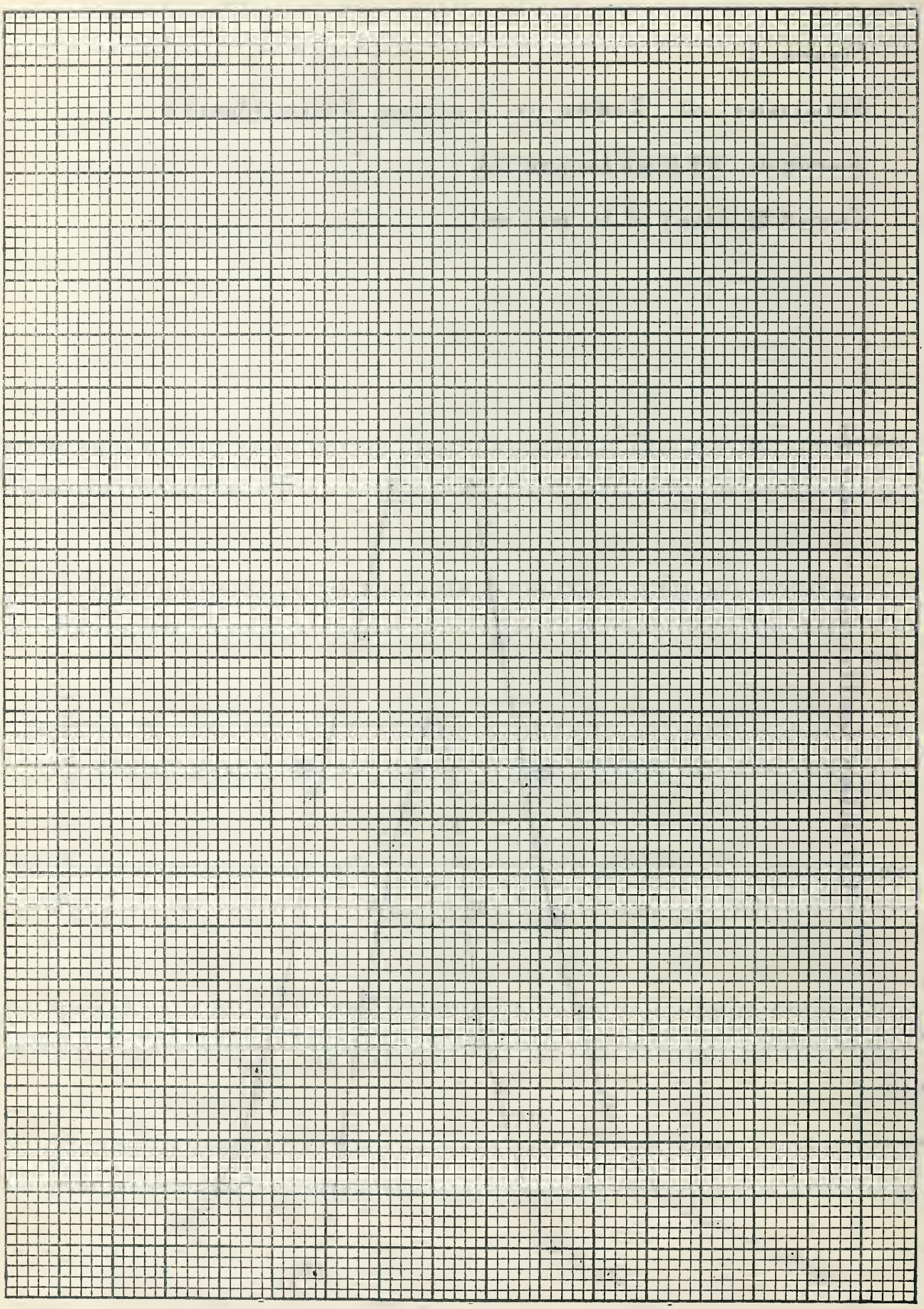
Figure 2

Solutions of Iodine in Carbon Tetrachloride

Curve ① 48 γ of I_2 per ml.

Curve ② 1800 γ of I_2 per ml.





Calibration of the Lumetron Photoelectric Colorimeter

Filter 515 supplied with the instruments was used for transmittancy measurements for iodine solutions. The sample holder is a fused glass cell 1 cm. square having a volume of 1 ml. A cover slip which is held in place by the surface tension of the solution prevents evaporation of the carbon tetrachloride while the reading is being taken.

For the calibration, volumes of a standard solution of potassium iodide measured in calibrated pipettes were treated in the same way as the sample. The method is given below.

A measured volume of standard potassium iodide solution is added to 5 ml. of distilled water. A few crystals of sodium sulphite, a pinch of sodium azide and 2 ml. of 85% phosphoric acid are added. The solution is boiled for 2 minutes and cooled. It is then transferred to a graduated separatory funnel and the volume is made up to 25 ml. with distilled water. Then 2.5 ml. of carbon tetrachloride are added. A few crystals of sodium nitrite then oxidize the iodide to iodine which dissolves in the carbon tetrachloride to a violet solution. The carbon tetrachloride and water layer are mixed well by shaking to ensure equilibrium between the

iodine concentrations in the two layers. The cell is first filled with pure carbon tetrachloride and the galvanometer is set to zero by adjusting the angle of the balance photocell (Bc and Bf Figure (IV) Section I). The carbon tetrachloride is replaced by the iodine solution and % transmittancy readings are taken till they become constant. (Water droplets in the solution give an initial low percentage).

The data shown in Table II, are averages of three determinations except where a number in brackets before the logarithm of the percentage transmittancy designates otherwise.

TABLE II

Calibration Data

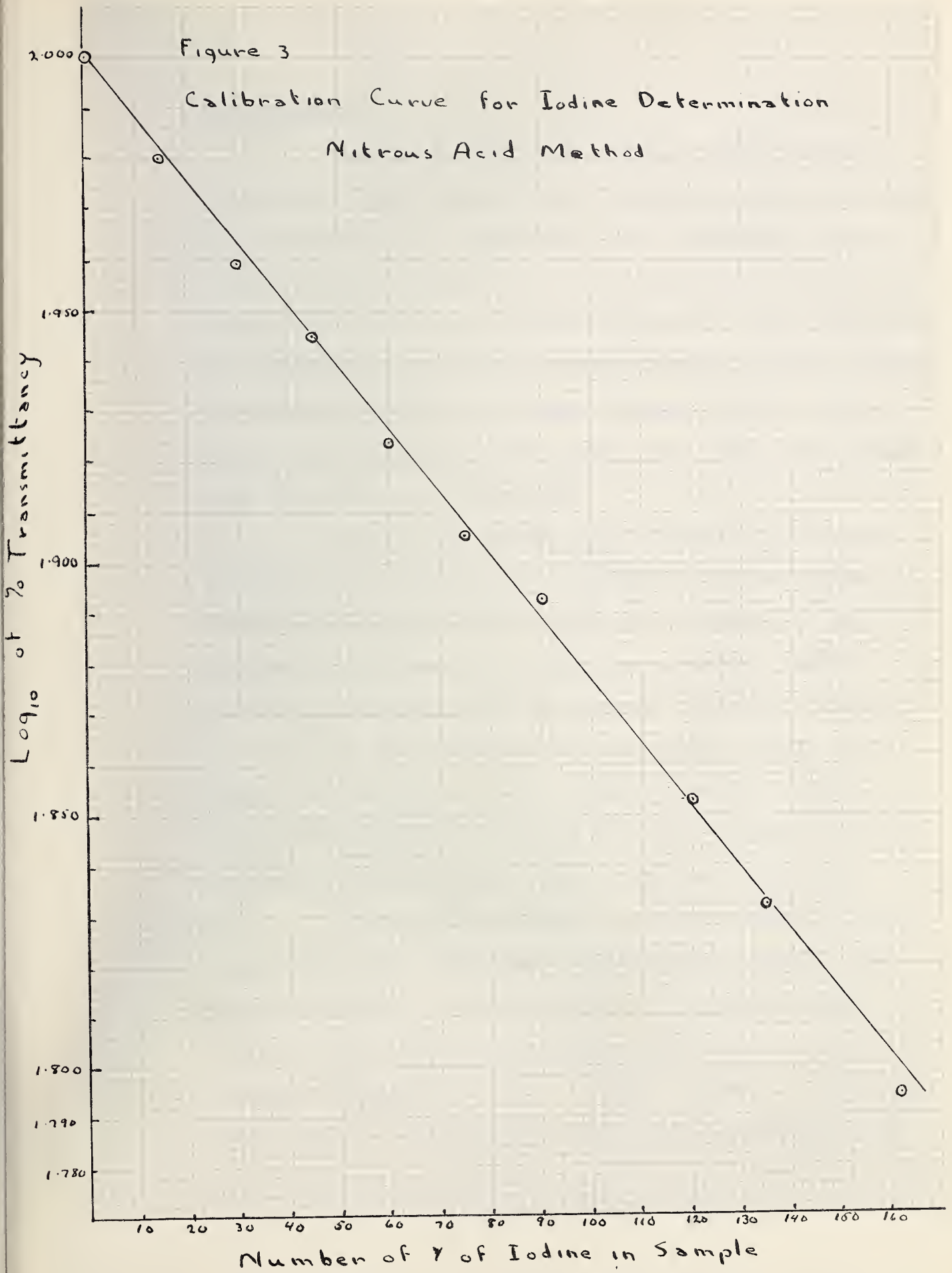
Iodine as KI used as sample (y)	Log_{10} of % Transmittancy
15	1.980
30	1.959
45	(2) 1.945
60	1.919
75	(2) 1.906
90	1.893
120	(2) 1.853
135	1.833
162	(2) 1.794

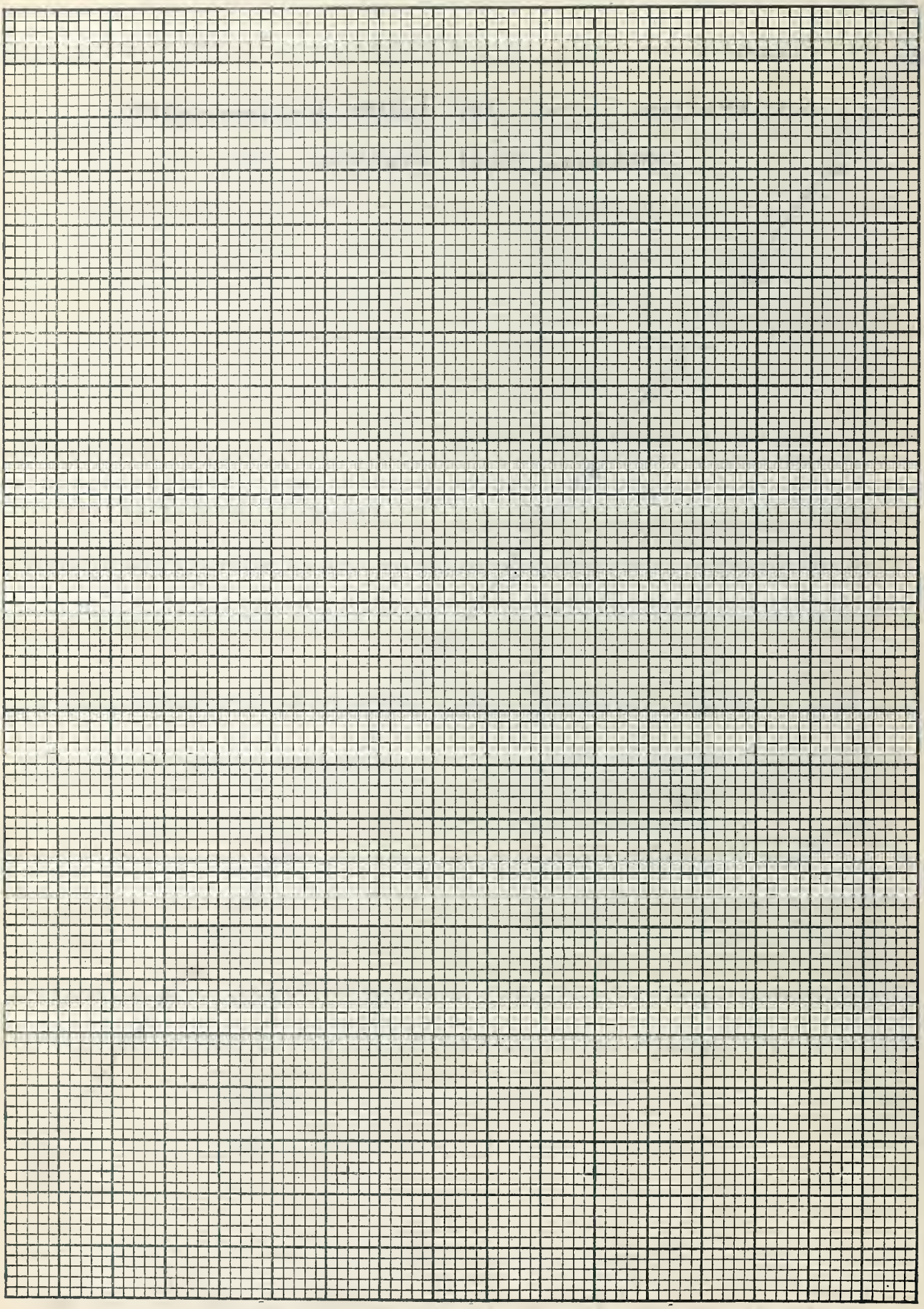
The points are plotted in Figure 3.

Figure 3

Calibration Curve for Iodine Determination

Nitrous Acid Method





Experimental

The torch method as outlined under "Methods of Analysis" was first tried. Difficulty was experienced in getting complete combustion. The tenacious ash protected the inner parts of the cartridge resulting in an inner core of partially burned material. This difficulty was overcome by placing one casing inside another, tying the end and putting the sample between the two. The casings are much more inflammable than the samples and a good combustion was obtained.

To test the method and to develop the proper technique small samples were burned in the varnished torch. Two samples of 50 grams were burned. To one sample a known amount of iodine as potassium iodide solution was added prior to burning. This same amount was added to the solution from the second sample just before the oxidation of the iodide to iodine in the colorimetric step. A failure to obtain a check on these duplicates would indicate that a loss was occuring.

First results showed a loss of iodine in both cases thus showing that some loss occurred during the process and some in the last step. The loss of the iodine in the last step was shown to be due to the presence of copper in the solution. Copper in solution, reacts on the addition of sodium azide as shown by the

equations below.

$Cu^{++} + e \rightarrow Cu^{+}$

$Cu^{+} + I^{-} \rightarrow CuI\downarrow$ pale yellow.

The solutions became yellow and slightly opalescent when sodium azide was added. Difficulty was encountered in keeping traces of copper out of the solution since the heat generated by the burning sample was sufficient to blister the varnish on the exposed copper parts of the torch. Thus some of the exposed copper was oxidized and entered the solution. Therefore attempts were made to overcome the difficulty either by removal of the copper or prevention of the reaction shown above. An attempt was made to remove copper by precipitation with an alkali carbonate. Enough copper, however, was carried through to interfere in the final step.

According to Hume and Kolthoff (5) sodium citrate when added to a solution of a cupric salt and iodide, forms a stable complex with the copper, so that it is not reduced to the cuprous form with sodium azide. Thus there is no formation of cuprous iodide. Attempted standardizations, however, using 5 ml. of 1.0 M sodium citrate (added before the sodium azide) gave erratic results so that no further work was done with the citrate.

In the official method for iodine adopted tentatively by the Association of Official Agricultural Chemists (2) the plant material is burned with copper oxide as an oxidation catalyst. Sulphuric acid rather than phosphoric acid is used and the solvent for final extraction is carbon disulfide rather than the tetrachloride. No mention is made of trouble due to copper. (Cuprous iodide is soluble in sulphuric acid.) It was thought that some adaptation of this would solve the copper problem here but the use of carbon disulfide was found unsatisfactory due to its volatility. Attempts to use a combination of sulphuric acid and carbon tetrachloride gave poor checks in work with known amounts of iodide. The only remedy therefore appeared to be the preventing of contamination of the solution with copper.

A better quality varnish was tried on the torch and was found to be more heat and alkali resistant but not sufficiently so to prevent copper from contaminating the solutions.

The torch was then plated with nickel on all parts exposed to the heat or to the absorption solution. This prevented copper from entering the solution. Duplicate runs still did not check however so it was decided to test for possible losses during the manipulation.

In order to dispense with the time-consuming combustion and evaporation pairs of 25 gm. samples with added iodide were ashed in an open dish. The ash was then leached with water and this solution treated as though it were the absorption solution of the torch method. Iodide was added to one sample at the end, just prior to the colorimetric step and to the other at various points in the procedure to ascertain the point or points at which losses were occurring. In most cases the ignition in the platinum dishes as described under the torch method was dispensed with since the initial ashing was complete and there is small chance of a loss of iodide during this ignition.

Results showed losses throughout the procedure. A summary of these results is shown in Table III

TABLE III

Loss of Added Iodide

Iodine added (γ)*	Point in procedure at which Iodide added	Average Recovery (γ)
150	Before ashing	110
150	Before leaching	120
150	Before alcohol ext'n	125
150	Before ignition of extract	140

* 1 gamma (γ) = 1 X 10^{-6} grams

Tests were made on the efficiency of the alcoholic extraction of potassium iodide from a mixture of potassium iodides and carbonates. The following points were noted.

(1) The alcohol extraction, using 150 cc. of alcohol was adequate for 150 gamma of iodine as potassium iodide when admixed with potassium carbonate.

(2) A yellow compound, soluble in alcohol was formed when the alcohol was allowed to remain in contact with the salts for more than 15 minutes. This yellow compound was decomposed by oven heating at 400°C for 15 minutes.

(3) The loss of iodine appeared to be proportional to the amount of yellow compound formed.

(4) The alcohol by itself left no residue when evaporated to dryness.

Since no organic matter other than alcohol was present it was thought that the colored compound was an organic iodide formed by reactions similar to those shown below (20).

$$CH_3 - CH_2OH + NaOI \rightarrow CH_3CHO + NaI + H_2O$$

$$CH_3 - CHO + 3\ NaOI \rightarrow CI_3 - CHO + 3\ NaOH$$

$$CI_3 - CHO + NaOH \rightarrow NaO\text{-}CHO + CHI_3 \quad \text{Iodoform}$$

It had been noted previously that a yellow brown compound was present in the residue from the alcohol extraction if the alcohol had been allowed to remain in contact with the salts being extracted for appreciable periods of time. Iodoform is yellow but the presence of persulfate imparts a red or orange color to it. (4) Since sulphuric acid is used to neutralize the absorption solution it is probable that the persulphate ion would be present. The yellow brown compound was found to be soluble in carbon tetrachloride to give a yellow solution as does iodoform.

Tests on iodoform showed that the iodine in it could be changed to inorganic iodide by boiling in concentrated solutions of sodium hydroxide. Therefore this step was incorporated into the method.

Test determinations were made using the nickel-plated torch. A treatment of the residue from the alcoholic extract with sodium hydroxide solution was incorporated into the method. Recoveries of added iodide using this modification were as high as 93 - 97% but there were frequent cases of complete loss. Because of these losses, the time required to burn a 200 gram sample (approximately 3 hours) and the care required during the combustion it was decided to abandon the torch method.

The Low-Temperature Ashing Method

Reports on the utility of this low temperature, open dish ashing method do not agree. Reith (15) reports large losses of added potassium iodide but good recoveries of iodine added in the organic form. Kolnitz and Remington (17) report good recoveries of potassium iodide added to thyroid extract. Karns (15) claims good recoveries of potassium iodide. These conflicting reports seemed to indicate that perhaps a method could be devised to determine iodine without the use of the torch. This low temperature ashing method has the advantage of simplicity and was considered to be better suited to the analysis of the large numbers of samples required in an iodine survey.

Critical Examination of the Low Temperature Ashing Method

An examination of the low temperature ashing method was undertaken, with the purpose of ascertaining steps in the procedure in which losses might occur. Conclusions and suggested improvements are listed below.
(1) Cereal grains yield an acid ash and to prevent the volatilization of iodine some form of alkali must be added. Intimate contact between the alkali and the particles of grain is necessary. It was found advisable

to double the quantities of calcium lactate and sodium carbonate added to the sample. Since solutions of calcium lactate deteriorate this compound was added as a powder, mixed dry and moistened when in the porcelain dish used for the combustion. It was further found that the addition of a saturated solution of sodium hydroxide in 95% alcohol to the sample reduced the time necessary for obtaining an ash of uniform grayness.

(2) Difficulty was encountered in controlling the preliminary charring using a flame. For this reason the samples are placed in an oven (constructed in this laboratory) which is in a fume closet. The door to the oven is left open until the samples are charred. It was found that a more complete combustion with less loss of added iodide could be obtained by using a lower oven temperature for longer heating periods. The maximum oven temperature was approximately 400°C, near the heating element (floor of oven) which decreased to 350°C near the tops of the evaporating dishes. Twenty to twenty-four hour heating periods were found to be sufficient.

(3) To prevent the probable interaction of iodate and iodide in acid solution and in the presence of carbon it was decided to leach first with distilled water and decant this to remove soluble compounds. The ash and

filtrate were then treated separately with sodium sulfite (to reduce iodates to iodides) in phosphoric acid solution. The sodium azide and bisulphite were added before the phosphoric acid. Filtration removed insoluble phosphates of heavy metals.

(4) It was found that better alcohol extractions were obtained with more ease by using a two phase liquid system. Therefore the evaporation of the solutions from the leaching step were evaporated nearly to dryness and transferred to a mortar where any crystals formed are ground to form an even paste before alcohol is added to extract iodides. All iodine must be in the form of iodides as iodates are insoluble in alcohol.

(5) The alcohol extract was evaporated to dryness at 45 - 50°C. It was shown that some organic iodide (probably iodoform) is formed during an alcohol extraction under conditions encountered in the determination. Since these organic iodine compounds sublime at rather low temperatures it was decided not to use a temperatures over 50°C to hasten the evaporation.

(6) It was necessary to find a method of conversion of iodine organically bound (from the alcohol extraction) to inorganic iodide. Boiling for 5 minutes in a concentrated solution of sodium hydroxide converted 90 - 95% of the iodine in iodoform (prepared electro-

lytically and recrystallized from 95% ethyl alcohol) to the inorganic iodide. A step including such a treatment was interposed between the evaporation and the ignition of the alcohol extract.

(7) The residues from the step described above were often colored. The colored organic compounds must be removed before the colorimetric step since they interfere by dissolving in the carbon tetrachloride or by causing the formation of a stable emulsion between the two layers. It was found that an ignition of these salts in nickel crucibles at 300°C for 30 to 60 minutes removed the colored compounds. McHargue (10) recommends ignition at 300°C for 60 minutes for best results.

(8) The galvanometer supplied with the photoelectric colorimeter was found to be sensitive enough to warrant reading the percentage transmittancy to the nearest 0.5%. This corresponds to a possible error of 4 to 5 gamma of iodine which when transformed to parts per billion becomes 20 to 25 parts per billion when a 200 gram sample of grain is used. Since cereal grains, expecially wheat, from low iodine regions often contain less than 20 parts per billion this sensitivity is too low. Therefore the iodic acid colorimetric method was tested. In this indirect method bromine is used to

oxidize iodide to iodate; then iodide is added to react with the iodate in the presence of acid.

$$5\ KI + KIO_3 + 3\ H_2SO_4 \longrightarrow 3\ I_2 + 3\ K_2SO_4 + 3\ H_2O$$

Six times the amount of iodine in the original is released.

Some workers regard the method with distrust (17). It is claimed that there is too much uncertainty regarding the presence of oxidizing substances other than iodates in extracts of the ash of plants. The procedure is also claimed to be unreliable because of the effect of atmospheric oxygen on free hydrogen iodide. More recent work (3) has, however, demonstrated the reliability of the iodic acid method in this application. Experiment has shown (3) that the indirect iodic acid method is as reliable as the direct nitrous acid method and has the advantage of causing a deeper color in the carbon tetrachloride. This means that a greater accuracy may be obtained or that the size of the sample may be reduced.

Tests on the method using known amounts of iodide showed that the points for the plot of the logarithm of the % transmittancy against the number of gamma of iodine fell on the same straight line (Figure 3) as was obtained by the direct method. The results of

these tests are presented in Table IV and plotted in Figure 4.

TABLE IV

Test of Indirect Iodic Acid Method

Iodine in Original (γ)	Log_{10} of % Transmittancy	Iodine present from Fig.3 (γ)	Iodine calculated (6 X original
15	1.890	89	90
20	1.853	119	120
22.5	1.833	135	135
27	1.798	162	162

The determinations in Table 4 are averages of triplicate determinations.

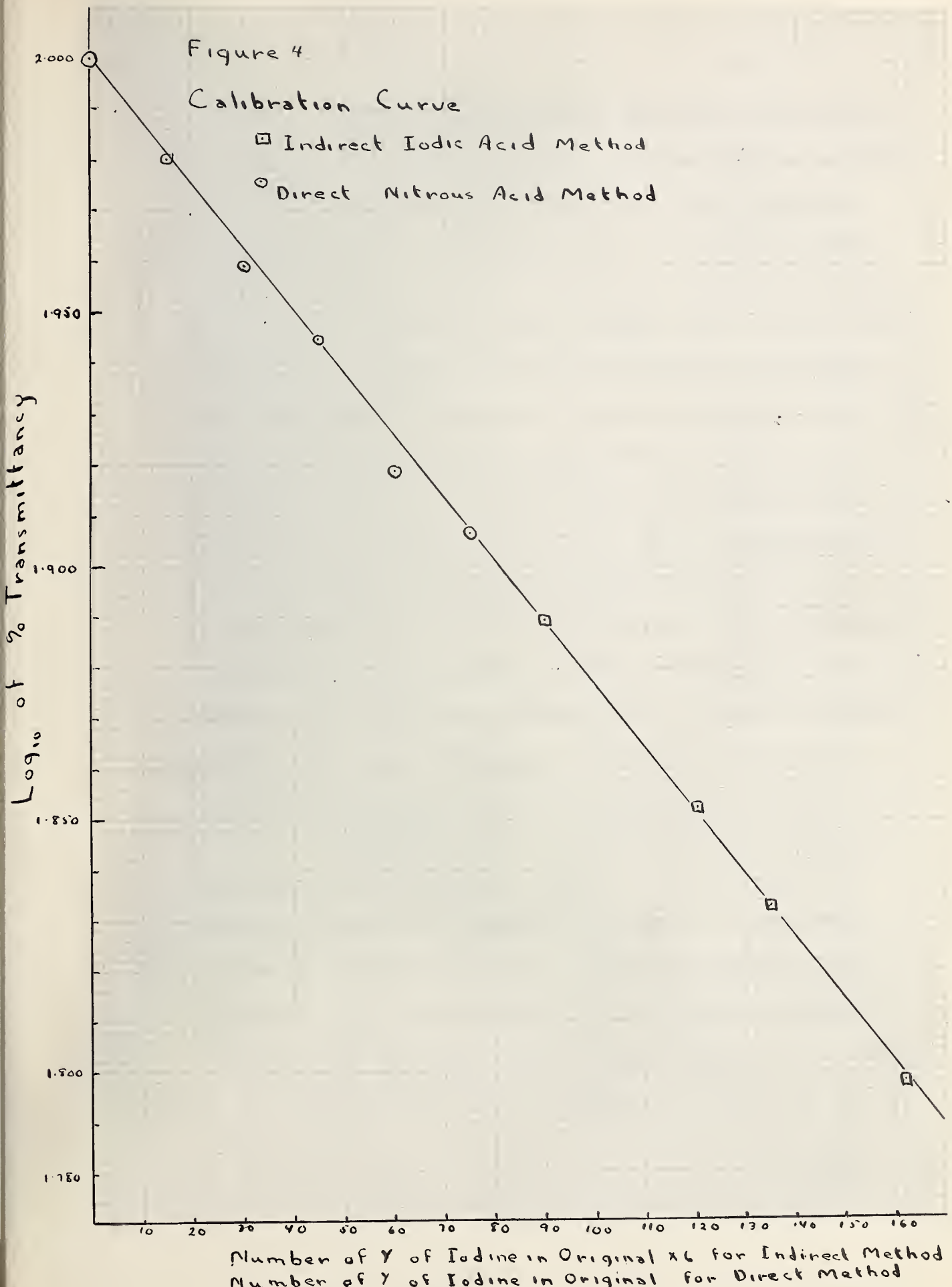
The indirect iodic acid method was adopted in place of the direct nitrous acid method. It is to be noted that the amount of iodine from % transmittancy measurements using this method is six times the amount originally present in the sample.

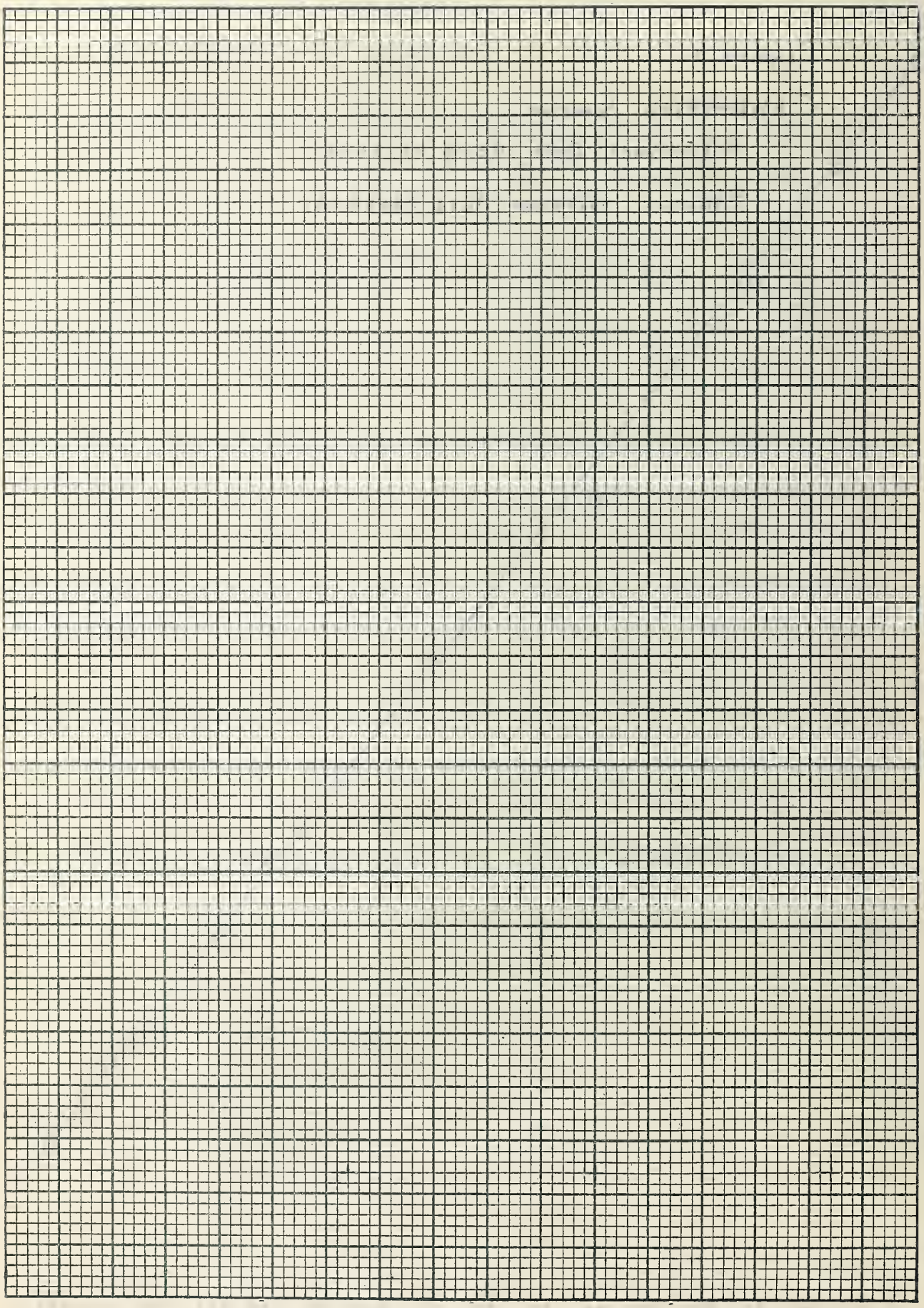
The Modified Method

The method finally used was one patterned after the low temperature ashing method and including modifications suggested by the work described above. An outline of this modified method follows.

Figure 4

Calibration Curve





Four hundred grams of the cereal grain are weighed out in 100 gram lots. To each 100 gram portion are added 2 grams of calcium lactate and 2 grams of sodium carbonate. The salts are well mixed with the grain and the mixture is placed in a porcelain evaporating dish. Distilled water is added till a thick paste is obtained. This is then dried and the four dishes are labelled - 200 grams (2 dishes) constitute one sample. To one sample a known amount of iodine as potassium iodide solution is added (one half to each dish of the sample). Then 50 cc. of a saturated solution of sodium hydroxide in 95% ethyl alcohol are poured over the contents of each dish. The dishes are placed in an oven at 350 - 400°C and heated for 20 - 24 hours. The oven is in a fume closet to dispose of the smoke. The ash remaining in the dishes should be a uniform light grey color when ashing is complete.

Distilled water is added to the ash in the dishes, the ash is stirred and the solution of the soluble compounds is decanted through a filter (both parts of the one sample are poured through the same filter). The ash is ground in a mortar and more distilled water is added. To the water and ash are added a few crystals of sodium sulphite and a pinch of

sodium azide. Then 85% phosphoric acid is added drop by drop until effervescence ceases. Sodium carbonate is added until the solutions are basic (a pinch of sodium carbonate fails to effervesce in the solution). The filtrate from the first decantation is treated in the same manner. Both solutions are now basic. The solution is decanted from the ash through the filter and the ash is finally washed with hot water. The total volume of the filtrate and washings should be approximately 200 cc. This solution is evaporated nearly to dryness in a large beaker and transferred to a mortar where it is ground and extracted 4 times with 50 cc. portions of 95% ethyl alcohol. The salts are best extracted when a two phase liquid system is used as noted by Reith (15). The alcohol is decanted through a filter and evaporated to dryness at 45 - 50°C.

Five ml. of distilled water and two pellets of sodium hydroxide are added to the residue and the solution is boiled for 5 minutes. The solution and rinsings are transferred to a nickel crucible (50 cc. volume) and evaporated to dryness. The nickel crucible and contents are then heated to 300°C for 30 - 60 minutes to char any organic matter present (10). The residue is taken up with water and filtered into porcelain evaporating dishes. The volume of solution is reduced to 5 cc.

Then the solution is carefully acidified with 6 N sulphuric acid to the acid point of methyl red. This is tested by applying a minute drop to filter paper soaked in an alcoholic solution of methyl red. The paper changes from orange to a deep red on contact with acid. Five drops more of the acid are added. Saturated bromine water is added until the solution acquires a permanent strong yellow color. This oxidizes the iodide to iodate. The solution is then boiled to remove excess bromine (disappearance of yellow color). A few crystals of salicylic acid are added to remove the last traces of bromine (9). The solution is cooled, transferred to a graduated separatory funnel and the volume is made up to 25 ml. with distilled water. Then 2.5 ml. of carbon tetrachloride are added. A crystal of c.p. potassium iodide causes the release of free iodine which dissolves in the carbon tetrachloride. The funnel is shaken 100 times to ensure the equilibrium between the two phases. Transmittancy measurements are made on the iodine solution using the photoelectric colorimeter with Filter 515. The instrument was calibrated according to the method given in the preceding section. The iodine calculated from the transmittancy measurements is six times the amount in the original sample. So results must be divided by six.

Results

In determinations using the method outlined above, iodine was added to one sample at the beginning and to the other just before the colorimetric step. Results for various samples and recoveries of added iodine are shown in Table V.

TABLE V

Iodine in Cereal Grains

Sample	Inorganic Iodide added (Y)	Organic* Iodine added (Y)	Recovery of added Iodine (Y)	Iodine in Sample (p.p.b.)**
W-1	15	0	14	36
B-1	3	0	2	135
W-2	15	0	13	25
B-2	0	18.4	18	6
O-3	3	0	3	95
B-3	0	18.4	19	85
W-4	2	0	2	8
B-4	0	0	-	30
O-4	0	0	-	30

* The iodine was added as an alcoholic solution of p - iodoacetanilide.

** p.p.b. = parts per billion parts of sample.

Conclusions

The results presented in Table V while not sufficient to definitely establish the method, indicate that it may prove useful. More tests and comparisons of results obtained by other methods are necessary. The method is of use for an iodine survey in which the iodine content of a large number of samples is required. The modified method is more rapid and as accurate as other methods which require expensive and fragile equipment.

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